ON THE LIFE-HISTORY OF LEMNA MINOR.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY.
XII.

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(WITH FIGURES 1–59)

THE greatly reduced sporophyte of the Lemnaceæ suggests the desirability of ascertaining to what extent these reductions have affected the gametophyte, and of looking more closely into the structures reported for the sporophyte. It is well known that flowering lemnas are seldom found, although during favorable seasons the vegetative plant is constantly seen in pools and slowly moving streams. Therefore, when in August 1897 an abundance of *Lemna minor* was found in flower it was decided to make an investigation of its morphology, in order to supplement previous work on the vegetative structures; to determine whether the usual sequences of monocotyledonous gametophytes and embryos were present; and to discover any suggestions of primitive or reduced conditions.

HISTORICAL.

The publications upon Lemnaceæ have been numerous, probably the most important being the monograph by Hegelmaier (4) in 1868, in which he gives the taxonomic features and also the general morphology in a way which is surprisingly accurate when one considers the condition of technique at that time. Another publication by Hegelmaier (2) deals entirely with the taxonomic features of the group; while in 1871 appeared a more detailed description of Spirodela (6) than that in the *Monograph*. Hegelmaier has also discussed the taxonomy of the group, based upon most recent knowledge (12). A similar work has been done for the American species by Thompson (16). Barbeck (8)

has described germinating seeds of *L. minor*, and shows that the "plumule" emerges from between the folded edges of the cotyledon; that the embryonic root is small and quite transient; that at the base of the "plumule," which persists but a short time, there appear quite early the young pouches in which new plants are developed vegetatively. The writer falls into a common error when he speaks of this vegetative reproduction as "an interesting case of parthenogenesis."

With reference to the time and conditions of flowering and forming vegetative shoots, Guppy (10) thinks that were temperature and moisture constantly at the optimum point no flowers would be produced. He suggests, further, that the "winter buds" are formed by plants which have been weakened by flowering. This does not accord with the cases reported by various other observers, who state that they are formed without the intervention of flowers.

Hegelmaier dealt sufficiently with the morphology of the sporophyte to show that it is practically reduced to a structure for the work of photosyntax; that part of this structure is so arranged as to form pouches which protect the vegetative buds and flowers; and that abundant air spaces, which float the plant, are formed by the separation or the breaking down of cell walls. By means of numerous figures he showed the history of the vegetative structures of the sporophyte.

None of the above authors investigated the gametophytes.

THE SPOROPHYTE AND ITS VEGETATIVE MULTIPLICATION.

According to the usual interpretation, the adult plant (*fig. 1*)[1] is a flattened stem divided into three regions, the basal stalk region (*fig. 1, a*) which represents the first internode, the nodal region (*fig. 1, b*) from which arise new shoots and flowers, and

[1] All drawings are from *Lemna minor*, and were made by means of Abbé camera. The magnifications given are those of the original drawings. Figs. 1–10 have been reduced in engraving to three-eighths and the remainder to one-half the original size. The stand and oculars used were by Reichert; the objectives were also by the same maker, except the $\frac{1}{12}$, an oil-immersion lens by Bausch and Lomb. The combinations used were as follows: × 35 (fig. 8), ocular 160^{mm}; objective 3; × 45 (figs.

above this another internodal region (*fig.* 1, *c*) which is entirely expanded as an organ of photosyntax. This function is also taken up by expanded portions of the node and basal internode, which are not distinguishable from the upper internode. In this

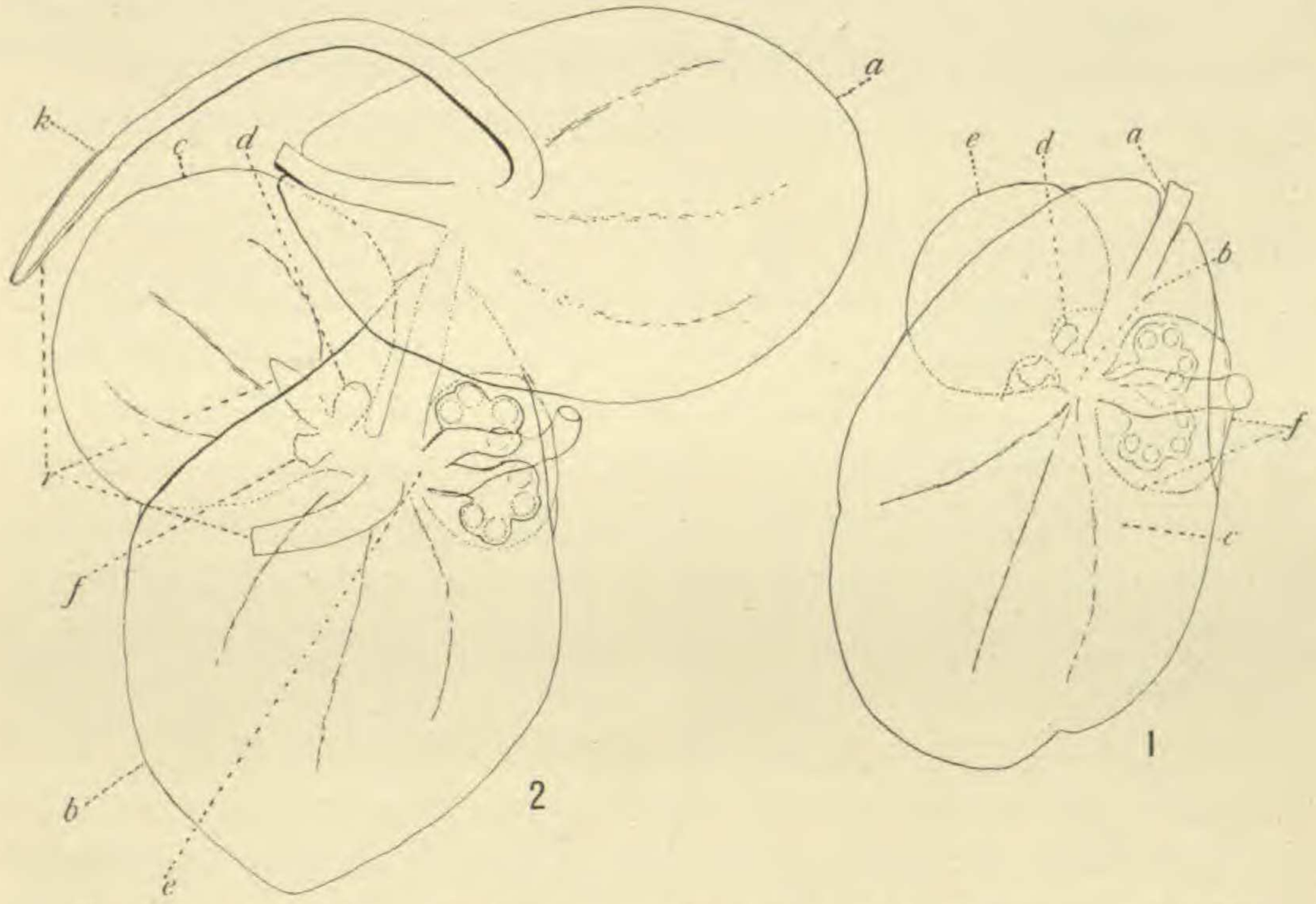


FIG. 1. Diagram of an adult plant. At the right of the main axis is a flower with its spathe, *f*, and carpel extending through the pouch opening. At the left, a young plant, *e*, bearing younger plant, *d*, opposite which is a young flower. *a*, basal internode. *b*, node. × 45.

FIG. 2. Four generations of plants, *a*, *b*, *c*, *d*, respectively. *e*, old flower. *f*, young flower. *r*, root. *k*, root cap. × 45.

upper region are three strands of conducting tissue, which in many cases were seen to proceed from the single vascular bundle that passes from the basal internode through the node. Frequently this presents the appearance of being one main axial bundle with a branch on each side. These conducting tissues extend almost to the margin of the frond,[2] passing in the upper

1, 2), oc. 2, obj. 3; × **73** (figs. 9, 12), oc. 4, obj. 3; × **260** (fig. 10), oc. 160^{mm}, obj. 7; × **520** (fig. 11), oc. 160^{mm}, obj. $\frac{1}{12}$; × **760** (figs. 4, 5, 6, 57, 59), oc. 4, obj. 7; × **890** (figs. 14–16, 42), oc. 2, obj. $\frac{1}{12}$; × **1375** (figs. 3, 7, 13, 17–37, 40, 41, 43–56, 58), oc. 4, obj. $\frac{1}{12}$; × **2400** (figs. 31, 32, 38, 39), oc. 12, obj. $\frac{1}{12}$.

[2] The term "frond" is usually applied to this entire plant. The use of the term

internode about midway between the dorsal and ventral surfaces. They are almost devoid of tracheary tissue, there being in the basal half of the strand a single row of very small tracheids, as shown by Hegelmaier. Throughout the entire strand there are two or three layers of phloem cells which are densely filled with protoplasm. The apical half of the bundle is composed entirely of such cells. It is quite probable that no tracheary tissue is needed, since the cells of the plant are all in direct contact with the water or nearly so.

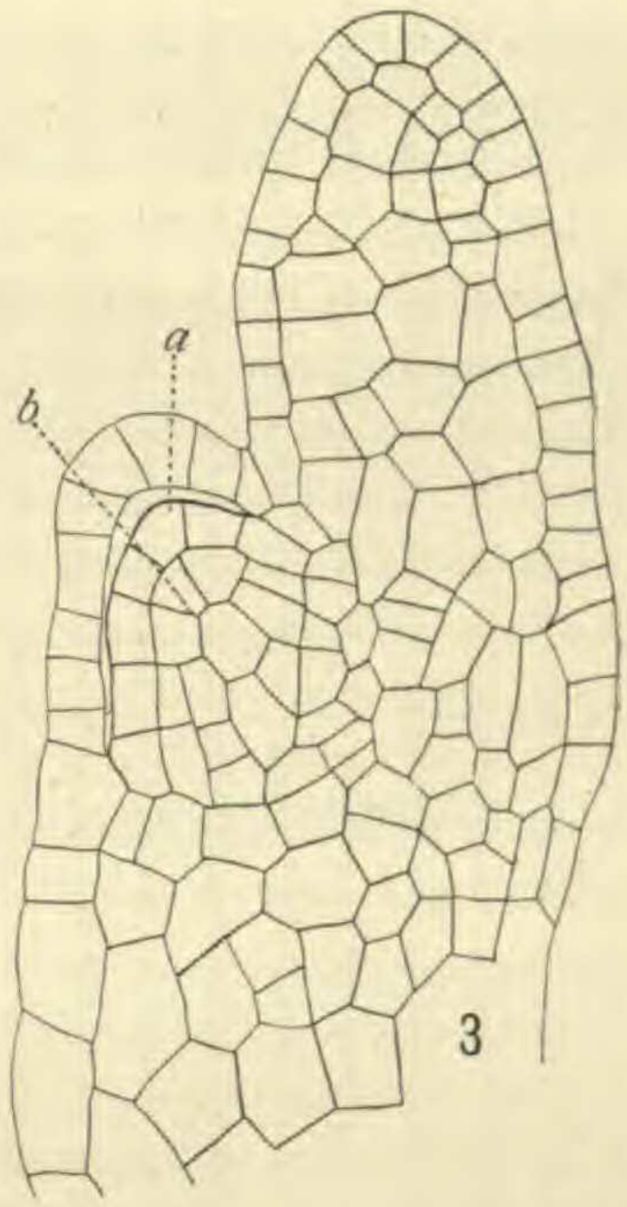
As described by Hegelmaier, the pouches in which the vegetative shoots appear are formed by outgrowths from the upper and lower surfaces of the parent plant. In an early stage the young plant appears in the bottom of the pouch as a very short-stalked outgrowth from the node (*fig. 1, d*), the mother plant in this case being about half grown and extending from the pouch of the plant *b*. My observations agree with those of Hegelmaier in that two young plants never appear at the same time on one side of a parent plant. While the bud is quite young its cells divide very rapidly, but soon cease such rapid multiplication and greatly increase in size. As they enlarge, the walls of the cells divide to form the air spaces, the latter being separated from one another by a single layer of cells. I was not able to find cases in which air spaces were formed by the breaking down of cells, as described by Hegelmaier. There are two general regions of intercellular spaces, the dorsal and the ventral, that are incompletely separated by a region of small cells through which the conducting strands pass. The cells about the air spaces contain very large chloroplasts which, as has been frequently mentioned, have unusual freedom of movement.

The rapidity of this vegetative multiplication is remarkable. At the time when a young plant extends half the length of the pouch in which it grows, it has itself developed pouches and begun a new plant. It is quite common to see attached to one

is unfortunate, since it was originally applied to the aerial part of the ordinary fern, which is morphologically quite different from the lemna plant. In the absence of a better term, and since I do not now wish to introduce a new one, the term "frond" may be used at times in this paper.

another four to six generations of plants, only one of which is fully formed. In *fig.* 2 there are shown four plants, two of which (*a* and *b*) are fully formed, one (*c*) is about half the adult size, and one (*d*) is a very young plant. With high magnification it would be possible to distinguish the beginning of a yet younger plant from *d*. The plants *b* and *c* each bear flowers (*e* and *f* respectively). *Fig.* 1 is from the ventral side, so that the roots (*r*) of the three older plants are shown.

The root arises from the lower side of



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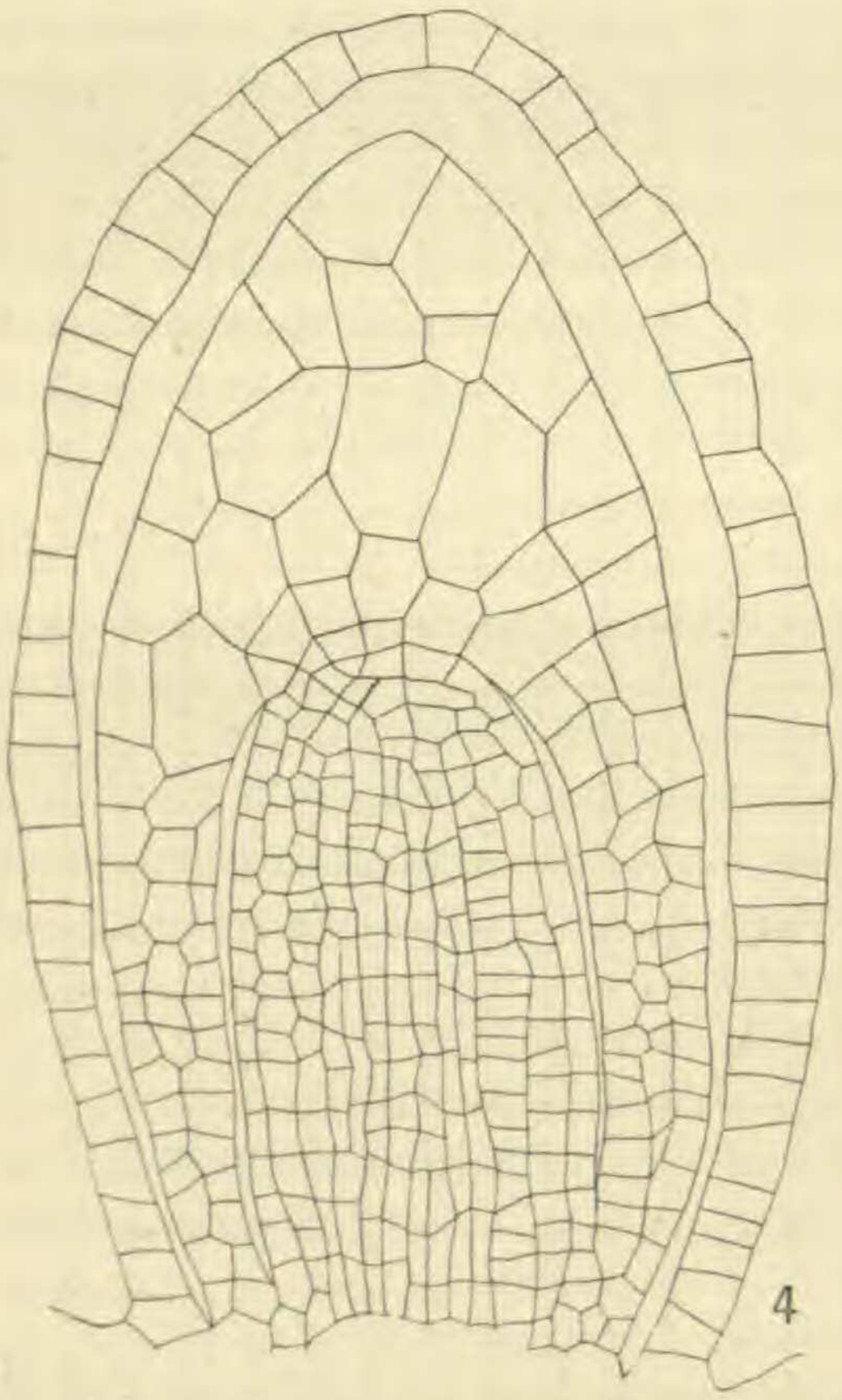

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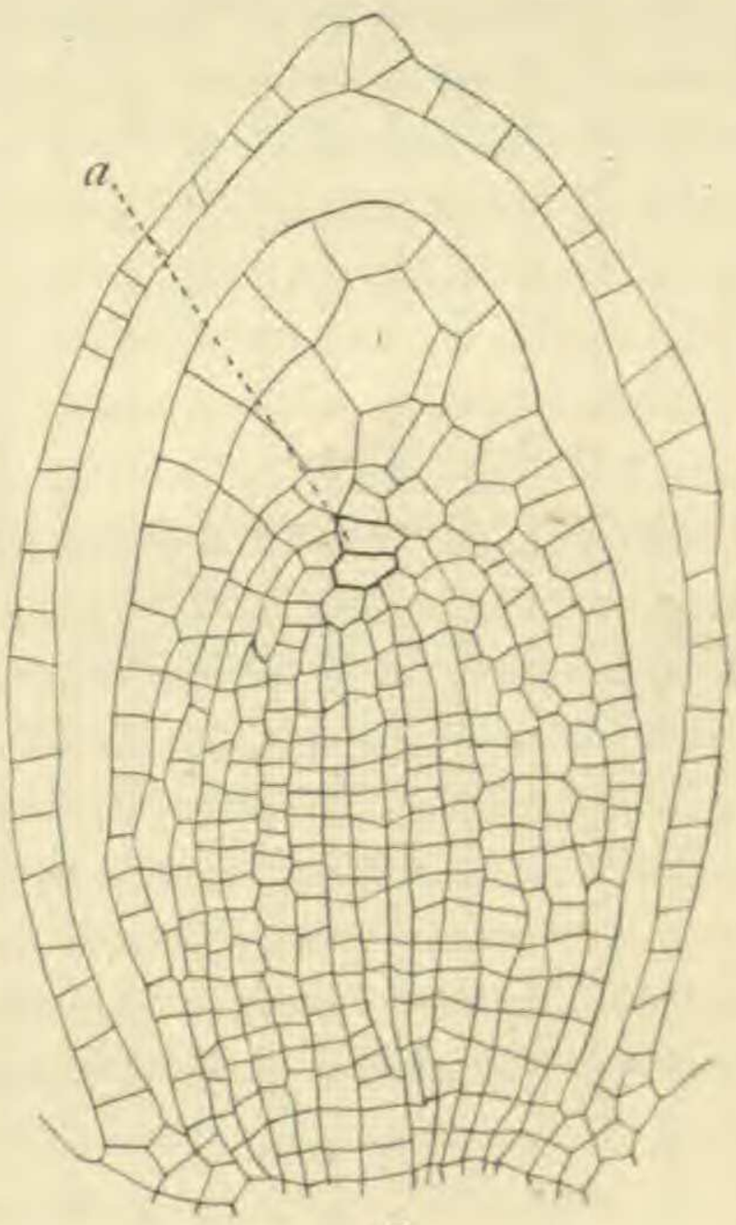

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FIG. 3. Young frond sectioned transversely to flat surface, showing young root below. *a*, root cap. *b*, meristem region. × 1375.

FIGS. 4, 5. Young roots, each showing temporary epidermal sheath, root cap, and the main body of the root. *a*, meristem cells. × 760.

the node. It may be distinguished quite early, appearing as a papilla formed by rapidly growing hypodermal cells (*fig. 3*). This figure represents a median longitudinal section cut transversely to the surface of the young frond. The epidermal cells divide as they are forced out, forming a temporary root sheath. This sheath may persist for some time (*figs. 4, 5, 6*) but is finally broken and decays.

Very early in the development of the root the layer of cells is seen (*fig. 3, a*) that develops into the root cap. This layer continues to thicken by additions from the meristem region below (*fig. 3, b*). The number of cells constituting this meristem region is unusually small (*figs. 5, a, 6, a*), in some cases consisting apparently of but one cell. The walls of these cells are drawn heavier than those of adjacent ones, simply to point them out. Miss Amelia McMinn (11) states definitely that this is an apical cell, and such may be true as to position, but probably not as to morphological character. I find no such break in the continuity of cells at the root tip as shown by Hegelmaier in *pl. 10, figs. 8, 9* of his *Monograph*.

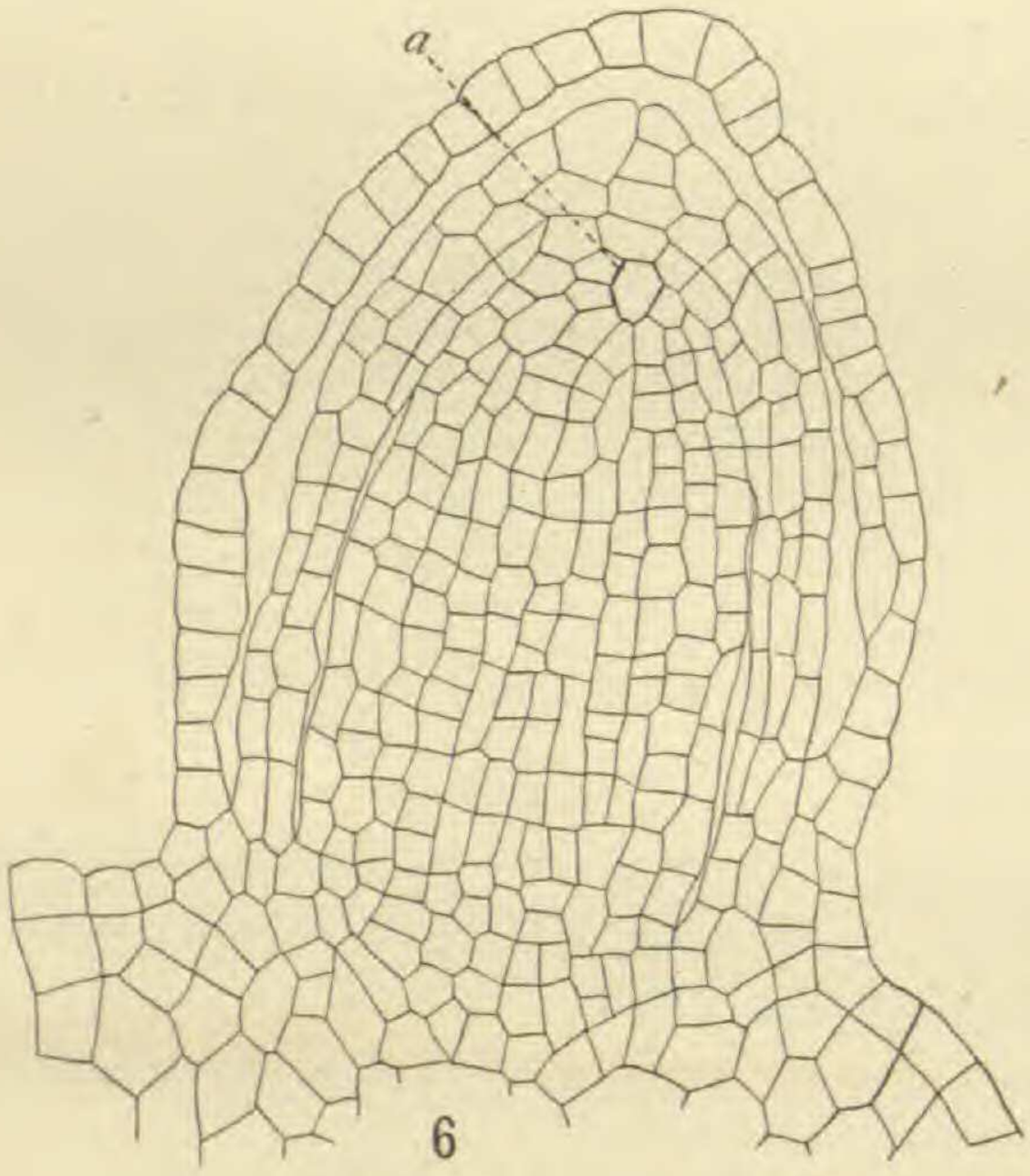


FIG. 6. Young roots, each showing temporary epidermal sheath, root cap, and the main body of the root. *a*, meristem cell. × 760.

The root cap is at first united with the main body of the root (*fig. 5*), but back of the growing point it may be seen quite early to become free, and before the root has attained any considerable length the cap has become separated from a region opposite to the meristem down to the base of the root (*figs. 4, 6*). In the older roots the cap is found still attached to the

growing point, and, although it has become free at the lower end, it remains closely appressed to the root (*fig. 2, k*). In *fig. 7* is shown a fairly young root in cross section, in which appears the temporary root sheath (*a*), the root cap (*b*), and the main body of the root (*c*), in which the axial region (*d*) is more or less separated from the cortical region by air spaces. The cells of this axial region, which are three or four times the length of the cortical cells and not so wide, are arranged with diagrammatic regularity about a central cell. The outer cells of this cylinder are seen to be dividing by periclinal walls. This axial cylinder is the representative of the conducting system of the root. The structure of the root is not unlike that of other water plants, *e. g.*, naiads and potamogetons.

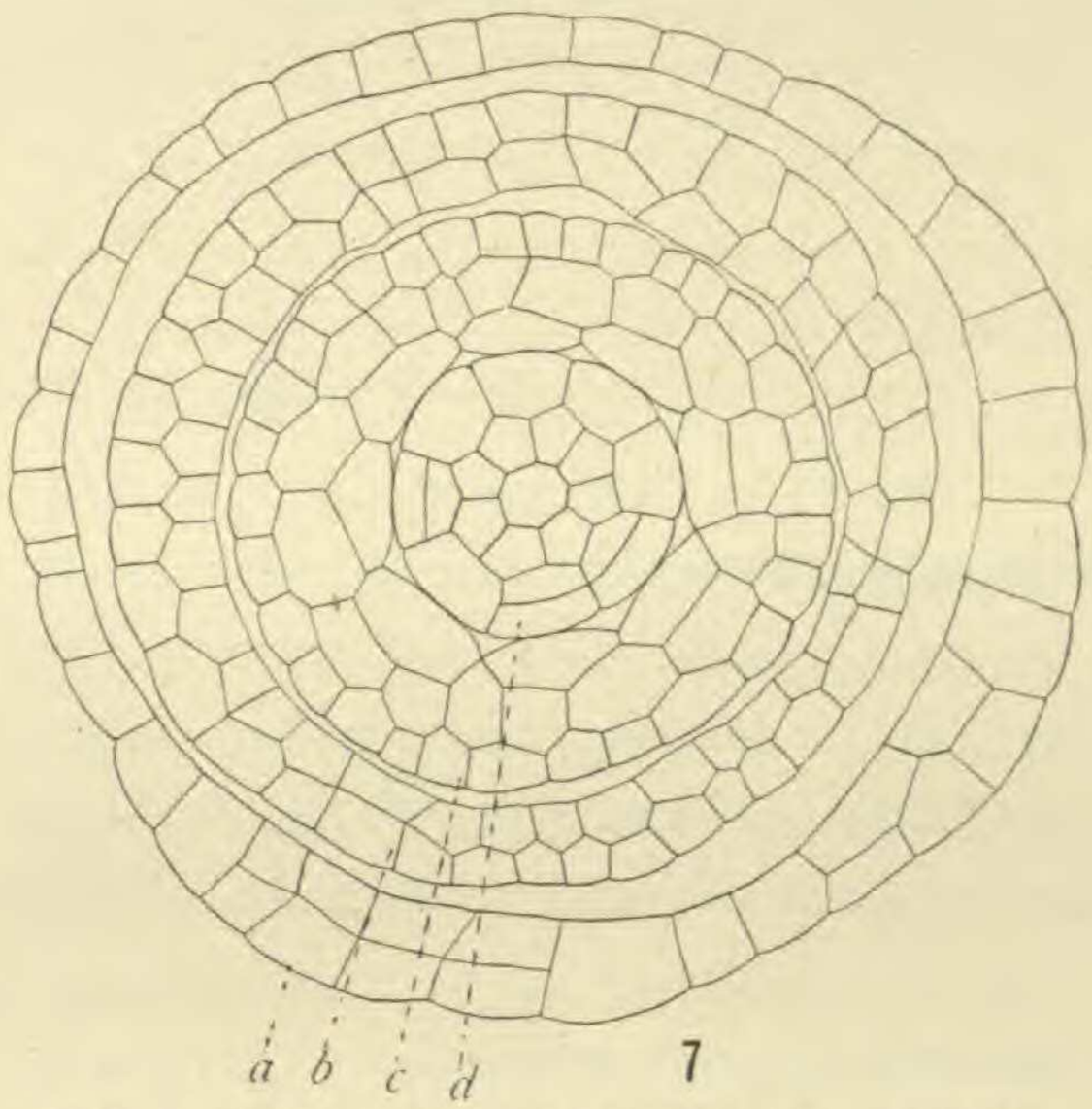


FIG. 7. Cross section of young root. *a*, epidermal sheath. *b*, root cap. *c*, body of root. *d*, axial cylinder. × 1375.

My own observations and those of others have led me to suggest that the lemna plant is not necessarily a flattened stem, in which the basal internode represents the original stem of the plant; or that it is a leaf with the power of producing new leaves from the specialized region known as the node; or that the basal region represents stem and the upper internode leaf; but rather that the entire structure is a shoot in which the basal and nodal regions are differentiated to serve special functions, and the upper internodes are entirely undifferentiated. The single conducting strand of the basal internode passes through the node and into the upper internode, where it may give off two branches, as in *L. minor*, or more, as in other species. It may also branch from the node into the new plants and the floral

organs. It appears that this strand is the axial bundle of the entire shoot, and that the apical region represents the undifferentiated condition from which the basal internode and node have become specialized.

With such an interpretation as this, the discussions whether this is flattened stem or leaf have no morphological basis, and it would be more appropriate to speak of the undifferentiated shoot as a thalloid structure than as a stem or leaf or frond.

ORGANOGENY OF THE FLOWER.

Plants in the flowering condition usually have well developed pouches at each side of the node. In one of these a young plant appears, while a flower develops in the other. This flower is often accompanied by a young plant, which when present appears just outside of the bottom of the spathe. Quite frequently it grows rapidly, and takes the place of the flower, though in many cases the parts of the flower are seen to have broken down before they were encroached upon, thereby making it clear that some other influence than pressure from the young bud injured the flower. Flowers may reach maturity, however, when young buds are growing at the side of the spathe.

In *fig. 8* is shown the diagram of a section made transverse to the surface of the plant, passing through the node in such

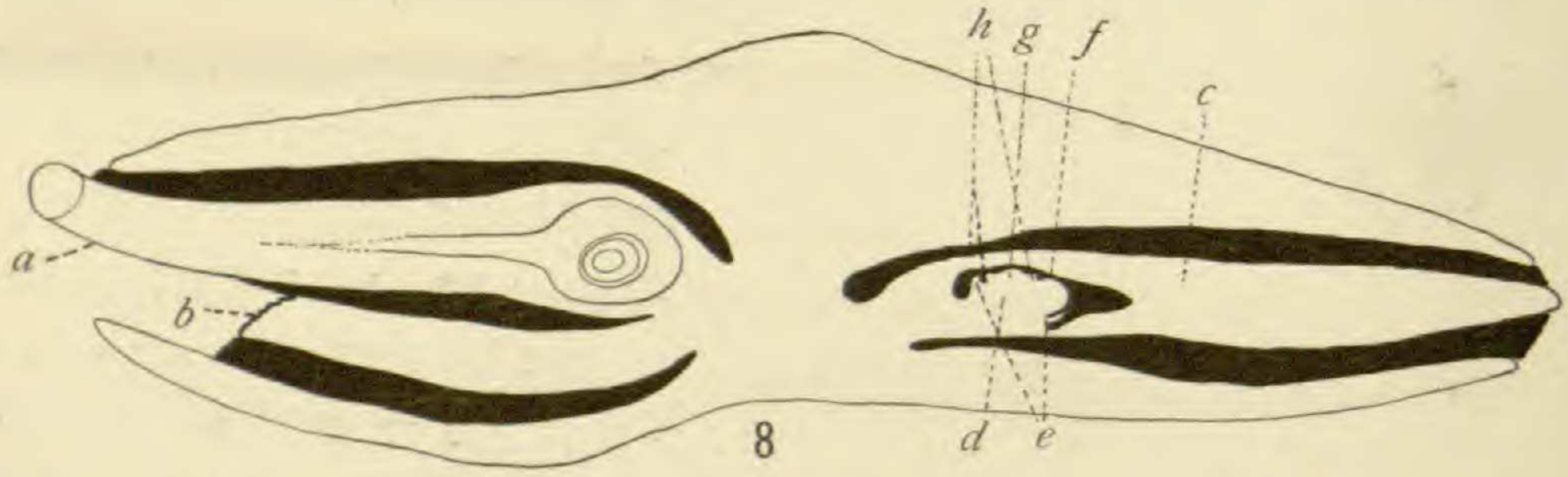
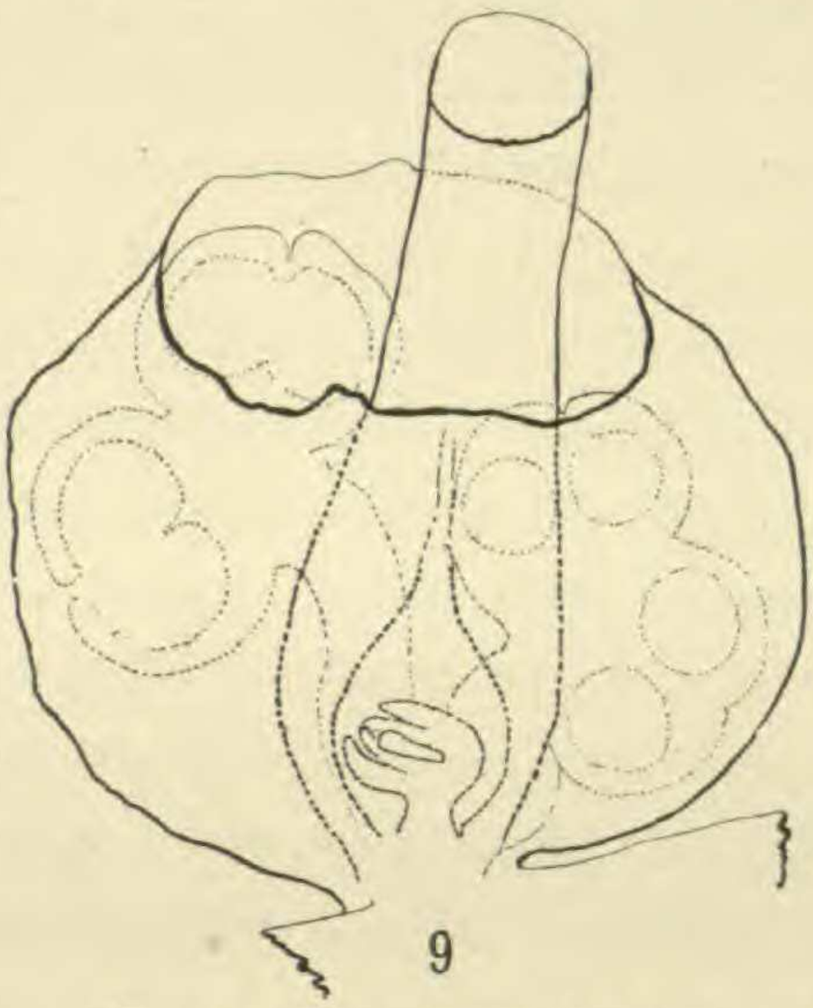


FIG. 8. Diagram of cross section of plant showing an old flower in one pouch and a young frond bearing a young flower in the other. × 35.

direction that in one pouch the carpel (*a*) and stalk of one stamen (*b*) of an old flower are shown; while in the opposite pouch is a young plant (*c*) which bears a young flower (*d*). At

the outside of this flower is the beginning of the spathe (*e*), which grows up about the other floral organs. This spathe when fully formed is usually one cell in thickness, though rarely it is two-celled at the base. It grows rapidly and soon extends beyond the edge of the frond (*fig. 1, f*), becoming the only means of detecting the presence of flowers when one is unaided by a magnifying glass. In *fig. 9* the fully formed spathe is shown as it surrounds the carpel and stamens.



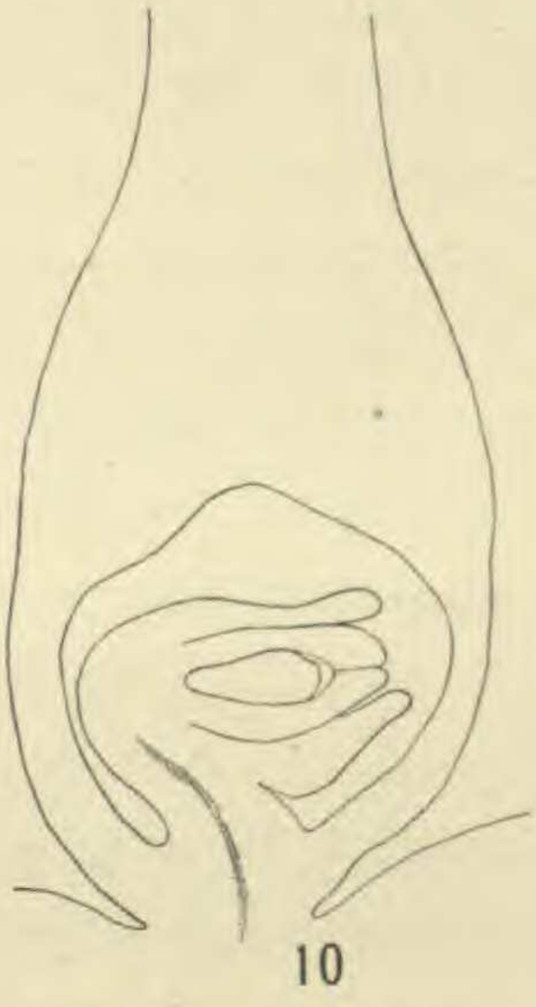
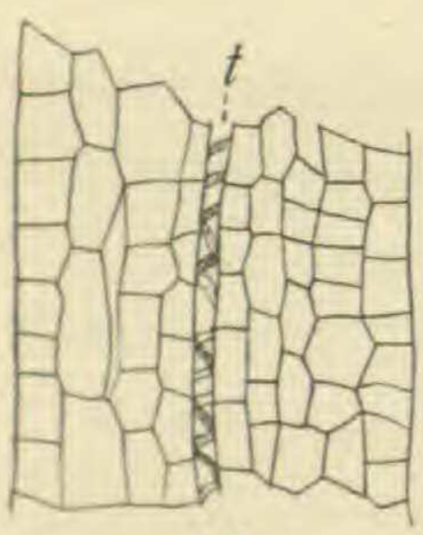


FIG. 9. Flower-spathe enclosing carpel and two stamens; ovule seen in carpel. × 73.

FIG. 10. Carpel with ovule; stigma region of carpel not shown. × 260.

The stamens appear at first as one small protuberance extending in the plane of the surface of the frond (*fig. 8*). As this projection becomes longer it branches, each branch later becoming one stamen. One of these branches is always less prominent and less advanced than the other. In adult stamens the single row of tracheæ (*fig. 11*) of each filament is seen to have originated from a common bundle at the region of branching (*fig. 12*). When the stamens are mature the anthers, the loculi of which lie in one plane, are pushed out beyond the margin of the spathe by the rapid elongation of the filament.



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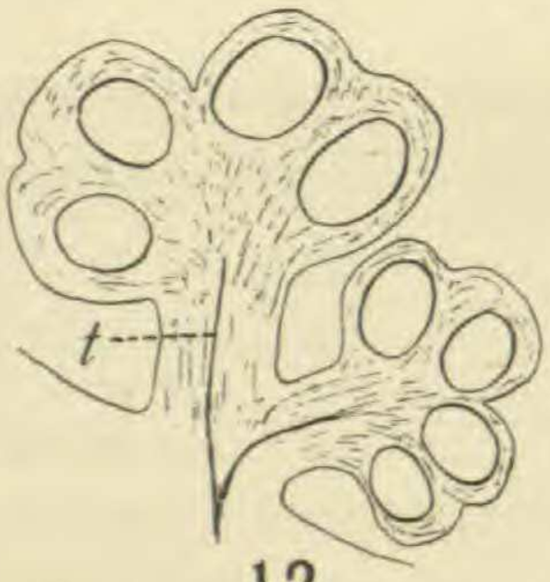

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FIG. 11. Longitudinal section of part of filament, showing single row of tracheæ, *t*. × 520.

FIG. 12. The stamens of one flower. *t*, vascular bundle. × 73.

The carpel arises from the same general region with the stamens. In *figs.* 8 and 9 is seen the nucellus, the projections on either side of which are to form the walls of the carpel. These projections grow rapidly, soon extending beyond the nucellus. A fully formed flower is shown in *fig.* 9, in which the carpel extends beyond the spathe and encloses the single half-anatropous ovule. Further discussion of the development of the carpel and ovule will be taken up in connection with the megaspore.

The homologies of the floral arrangement are very perplexing. It may be true, as given in the usual accounts, that there are here two flowers, one carpellate, the other staminate, enclosed in a common spathe. I can see no good reason for thus separating the carpel and the stamens. If the conditions are taken as they present themselves, it would seem as if this might be a single flower, with its parts probably spirally arranged, and enclosed within a spathe. If we are to take as evidence the supposed relatives of the Lemnaceæ, the aroids, we must recognize that the former are represented by forms so greatly reduced that we are safe in saying but little as to the kind of aroids from which they have been derived.

DEVELOPMENT OF THE MICROSPORES.

Young stamens which are not yet represented by distinct branches show a group of archesporial cells[3] immediately beneath the epidermis. One such stamen is shown in *fig.* 13. The two stamens at this time appear as obtuse outgrowths upon a very short stalk, the two together presenting the appearance of a single stamen in which two archesporial masses may be seen (*fig.* 14). Later stages show clearly that these are not two archesporial masses of one stamen, since as the anther grows each mass of archesporial tissue broadens and deepens and becomes divided by a plate of sterile cells which cease to take the characteristic stain of archesporial tissue. This is shown in

[3] While there may be some question as to just what cells should be called archesporial, for convenience I shall use the term in describing the cells of this region until they become clearly separated into sporogenous cells and those of the tapetal row.

the left of the two stamens in *fig. 14*. After a short time each of these groups of archesporial cells is divided in the same way, thus producing the archesporial masses of the four loculi of the stamen. This condition is shown in the older stamen of

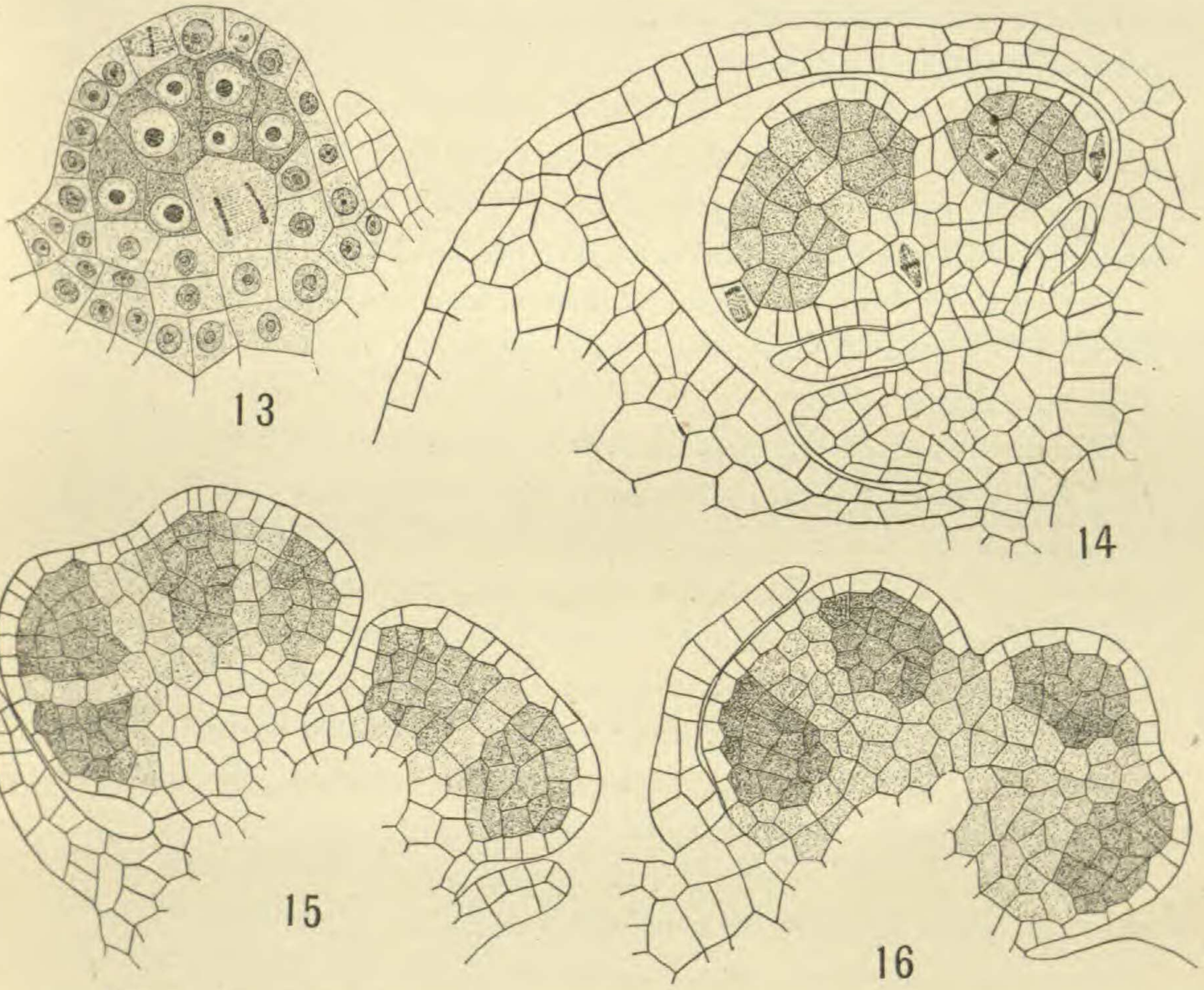


FIG. 13. Young stamen with archesporial tissue. × 1375.

FIG. 14. Two young stamens; in one the archesporial tissue is becoming divided by a sterile plate; beginning of the spathe is seen. × 890.

FIG. 15. Same as *fig. 14* but more advanced. × 890.

FIG. 16. One stamen showing the four loculi well separated. × 890.

fig. 15, while the younger stamen of this figure has its archesporium divided into two masses.

In an examination of later stages in the development of these tissues it will be seen that the separating region continues to develop new cells until the usual amount of sterile tissue between the fully formed loculi may be seen (*fig. 16*). In the

youngest anthers in which archesporial tissue could be detected, no indication of the exact region to become sterilized could be made out, since all the cells stain alike. However, one may soon distinguish the larger, more lightly stained, and less rapidly dividing cells which are to form the sterile plate. In *Naias flexilis* Campbell (14) observed that but one archesporial mass is formed in each anther; that this multiplies its cells for a considerable time before there occurs any differentiation into tapetal and sporogenous regions; and that "even in later stages the boundary between the sporogenous cells and those lying outside is not always perfectly clear." But in this mass of archesporial tissue no sterilized separating regions were formed, the entire mass forming a unilocular sporangium.

Such conditions suggest certain pteridophytes, where the sporangia are developed in a manner not unlike those of *L. minor*. In Isoetes we have an archesporial mass which, after having grown until it consists of a large number of cells, develops plates of sterile cells, the trabeculæ, which separate groups of sporogenous cells more or less completely from one another. In Isoetes the number and arrangement of these groups of sporogenous cells are irregular, while in *L. minor* they are regular; but in other respects the two present many points in common. The origin of the archesporial tissue seems to be the same, since in each it originates as a hypodermal layer; in behavior it is essentially the same, since in both it divides rapidly, forming a mass of cells, some of which become sterilized as plates which separate groups of sporogenous cells; and in each the tapetum is formed after these sterilized plates are completed.

If my observations and interpretations are correct, and if the spore bearing region of Isoetes is a single sporangium, the four groups of sporogenous cells of one anther of *L. minor* must be the same. Whether in angiosperms other than *L. minor* it can be shown that each loculus of the anther is not a real sporangium, as given in current accounts, but rather that two or four loculi of one anther are together the sporangium, must be

determined by further work. The question certainly deserves careful investigation.

Following the stage last cited (*fig. 16*), the archesporial cells continue to divide until two or three times the number of cells are developed. At about this time there appears immediately beneath the epidermis a layer of cells which by their reactions to stains are clearly differentiated from the cells beneath (*fig. 17*). Their nuclei stain more deeply, and the cytoplasm less deeply than the nuclei and cytoplasm of the cells beneath. This is the primary tapetal layer, while the cells below are the sporogenous cells, or probably by this time they are the microspore mother cells. The primary tapetal layer, therefore, is not cut off from the archesporium immediately following the appearance of the latter as a primitive layer, but after considerable masses of archesporial tissue are formed and segregated. It is not a primary but a secondary differentiation.

Between the mother cells and the epidermis in older loculi there are three or four layers of tapetal cells (*fig. 18*). The number of these layers is not necessarily regular for all the parts of a given loculus, as is shown in the figure just cited. I have not been able to decide definitely upon the origin of the tapetum. In some preparations the relative positions of the cells indicate that the tapetum has been cut off from the sporogenous cells, while in other cases it seems equally clear that it has come from the wall layers. It will be borne in mind that in the sporangia of the pteridophytes the tapetum is cut off from the sporogenous

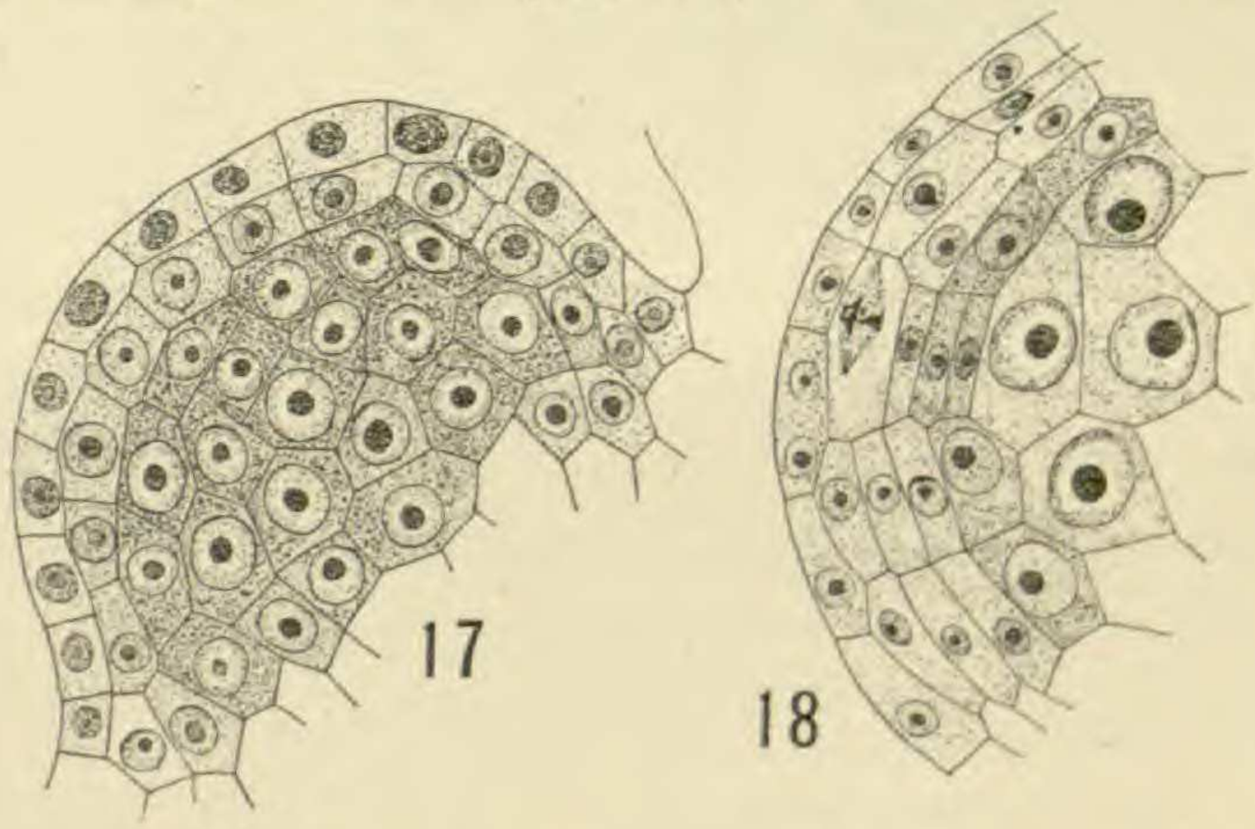
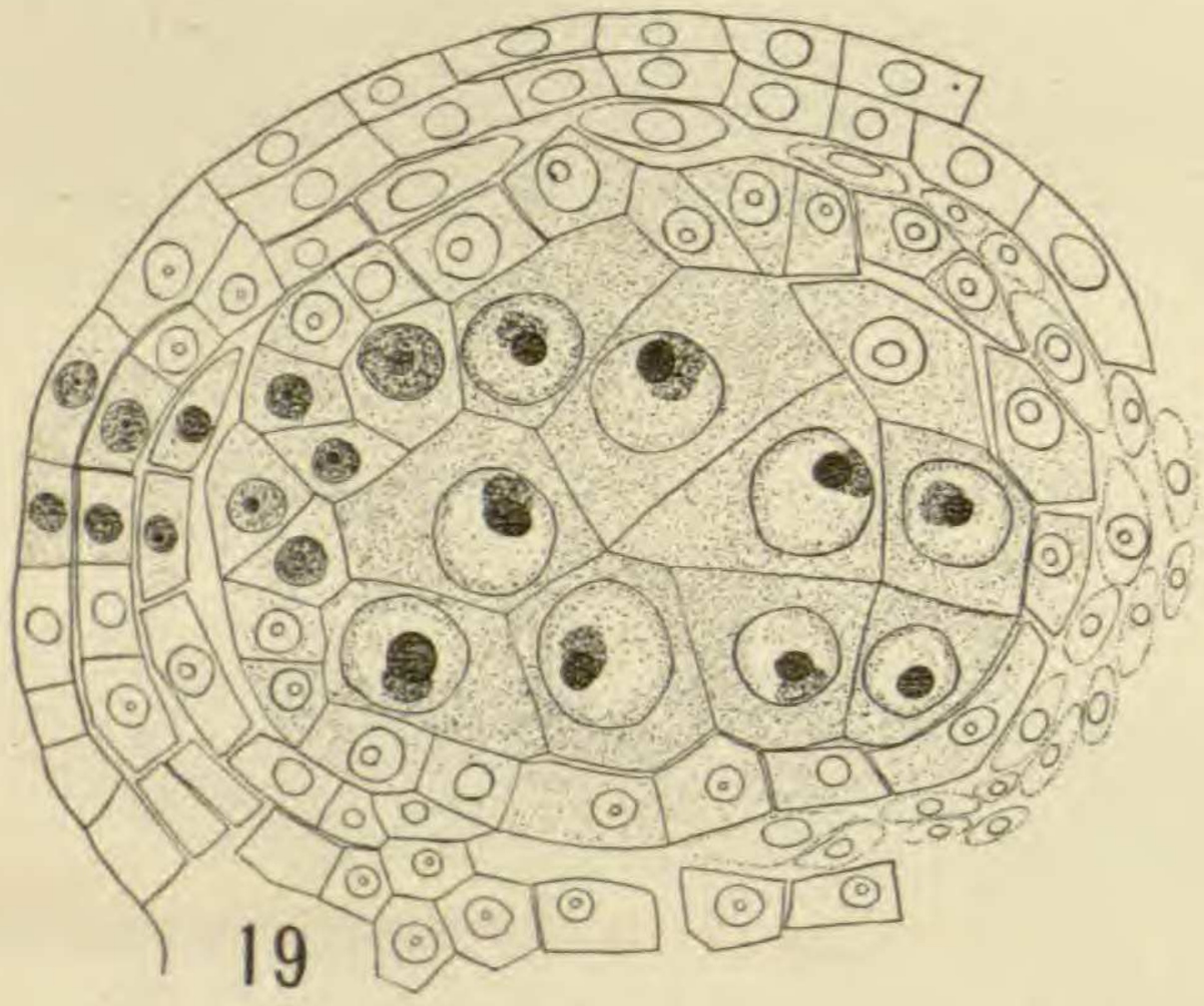


FIG. 17. A single loculus; tapetal layer differentiated about sporogenous cells. × 1375.

FIG. 18. Same as *fig. 17*, but advanced to a stage which shows several wall layers and the tapetum. × 1375.

cell, while in many composites it is equally certain that the tapetum is derived from the wall layers. Warming's account, reproduced by several text-books, refers the tapetum of angiosperms to the wall layers. So far as concerns the spermatophytes, the only evidence offered as to the origin of the tapetum has been the relation of its cells to those on each side of it. If, as appears to be true in this case, the tapetum may be derived either from the sporogenous cells or the wall cells, or perhaps partly from each, it would become clear that it is a physiological rather than a morphological layer. This accords with the conditions found in Ranunculus (17) and the suggestions made in that connection.

When the microspore mother cells have become free by the breaking down of their cell walls, the tapetum sometimes divides,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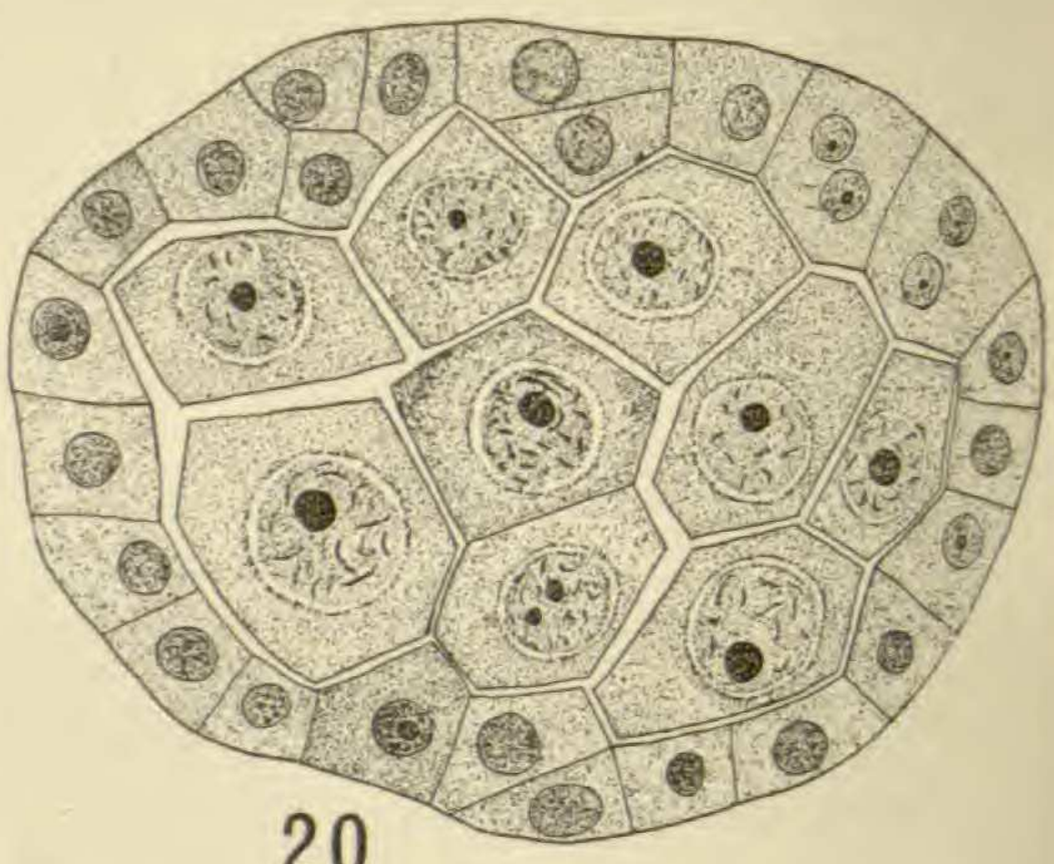
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FIG. 19. Loculus with mother cells, the nuclei of which are in synapsis; tapetal cells dividing; wall cells breaking down. × 1375.

FIG. 20. Same as *fig. 19*, with no wall cells. Nuclei in early stages of division. × 1375.

forming groups of cells projecting into the mother cell region (*figs. 19, 20, 21, 22*). A large number of cases were observed in which cells of the tapetum were projecting deep into the cavity of the loculus, a very peculiar case being shown in *fig. 23*. These projecting cells evidently served to nourish the mother cells, as the latter were frequently found in close contact with them, as shown in the figure last cited.

The number of microspore mother cells formed is as large as would be expected in a loculus of this size. A striking fact, however, is that comparatively few of them develop spores, since

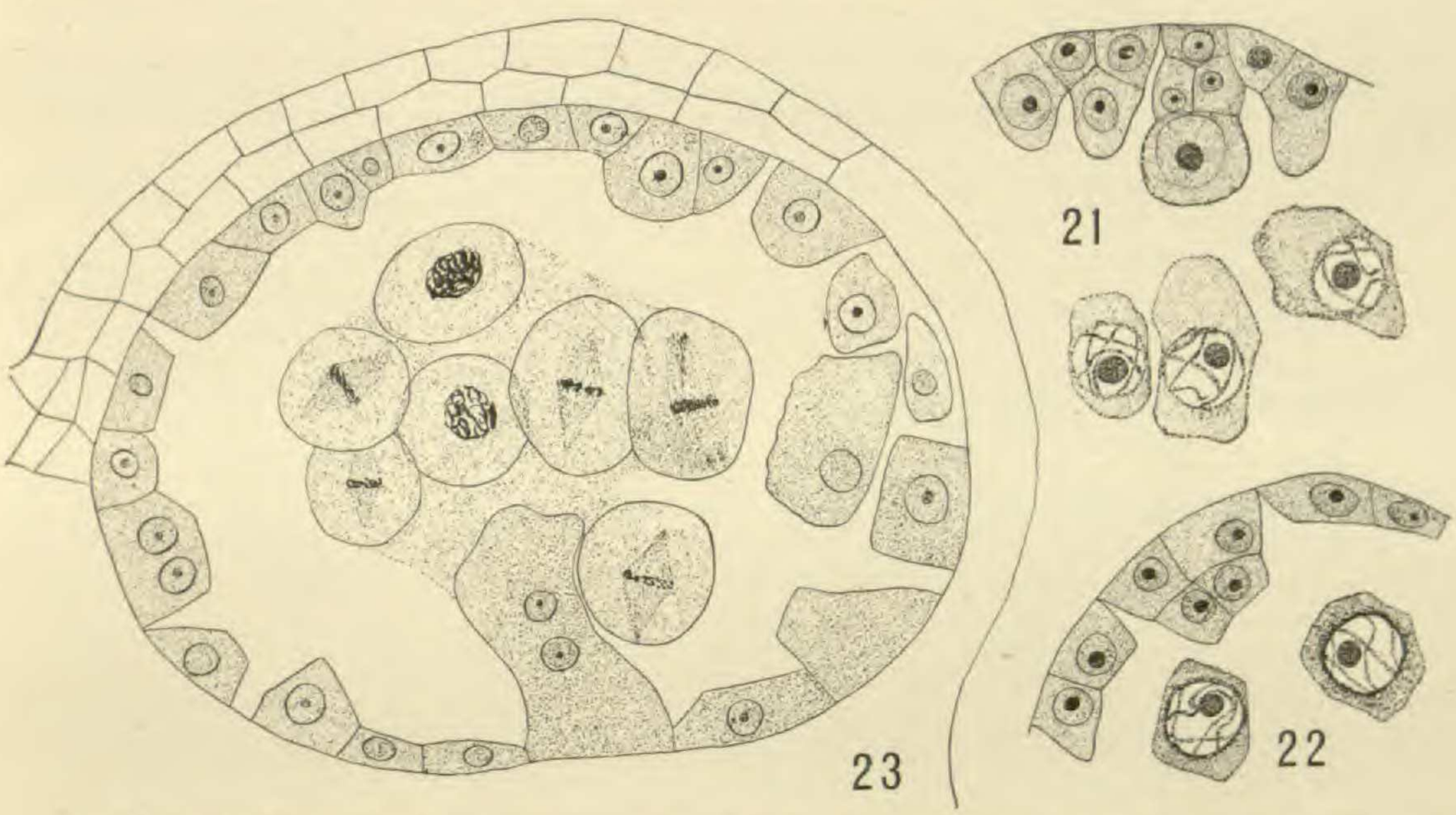


FIG. 21. Spore mother cells with distinct chromatin bands in nuclei, and irregular masses of cytoplasm about them; some tapetal cells divided. × 1375.

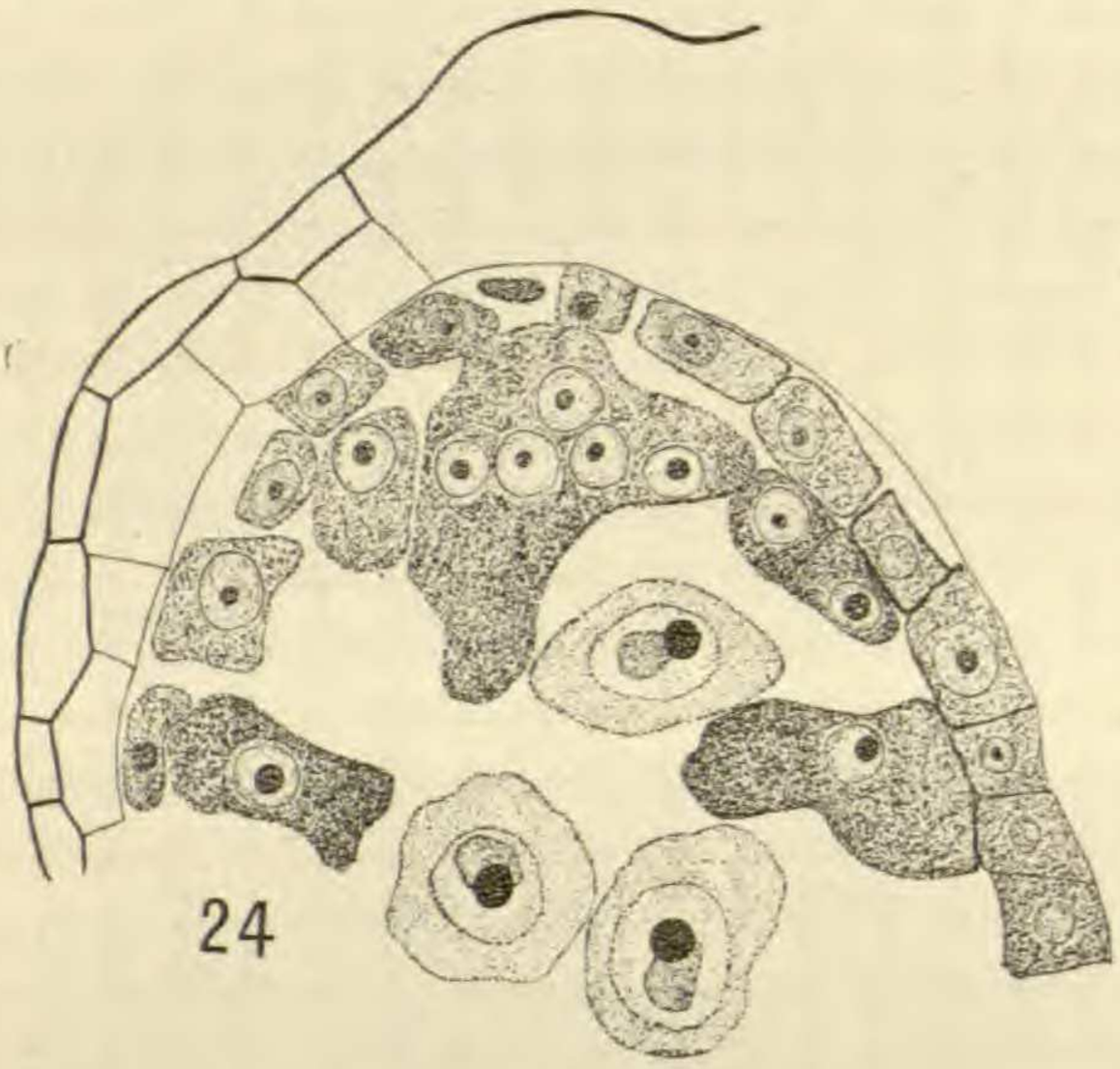
FIG. 22. Part of loculus; mother cells with divided tapetal cells. × 1375.

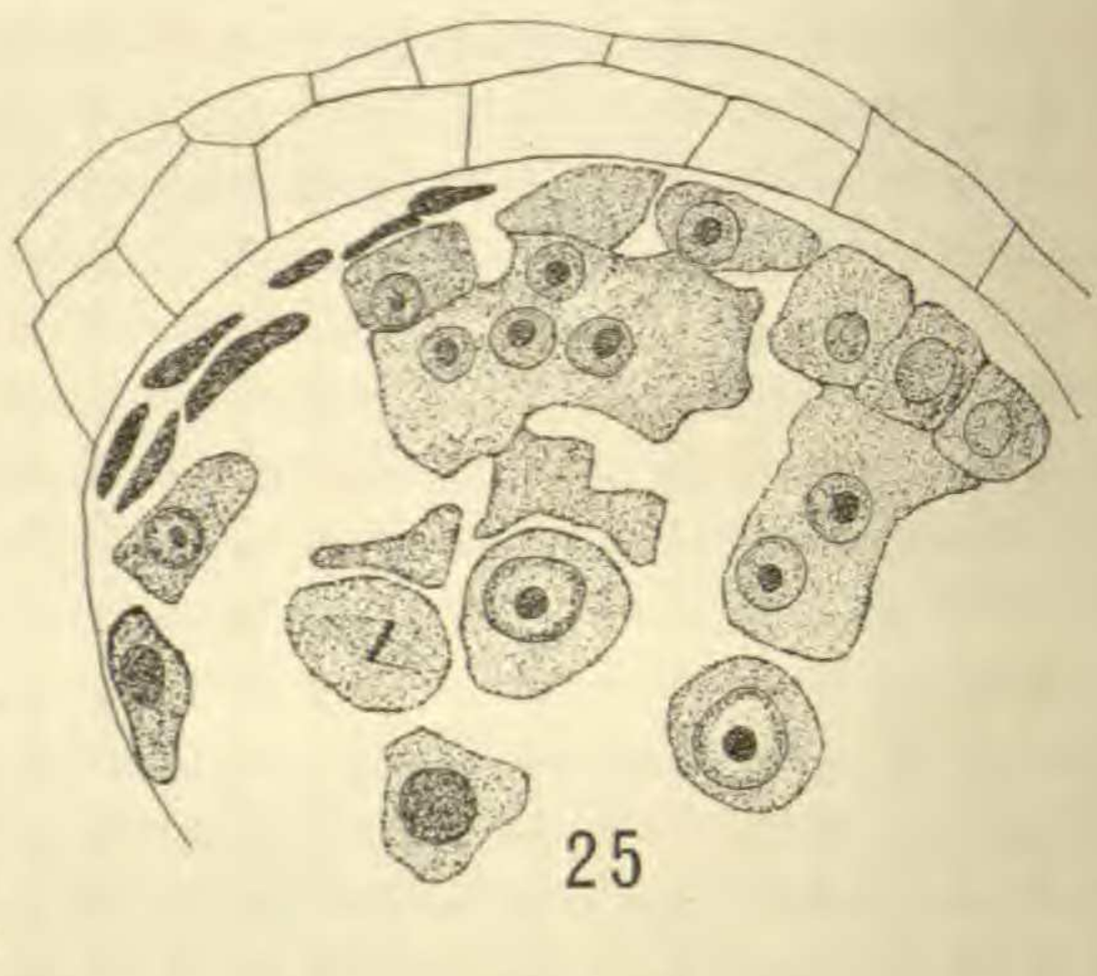
FIG. 23. Loculus with spore mother cells dividing; one tapetal cell has elongated very greatly, its nucleus having divided. × 1375.

many disorganize, and together with the tapetum nourish the remaining mother cells (*figs. 24, 25*). These broken down mother cells frequently form incomplete chains extending into and almost across the loculus, though such masses are usually found near the tapetum. They react to stains as the tapetum, and doubtless assume the function of the latter as nutritive tissue. Similar conditions are well known in Salvinia and Azolla, where disintegrating mother cells gather about the remaining ones and nourish them. In *Lilæa subulata* (18), also, there are certain cells between the sporogenous tissue and the tapetum which seem to represent those just described.

Each functioning mother cell gives rise to four microspores by first dividing into two divisions, each of these again dividing by spindles in the same plane as the first, but with their longi-

tudinal axes transverse to it (*figs. 23, 26, 27, 28*). Soon after these divisions have occurred the spores assume a spherical form and increase greatly in size.





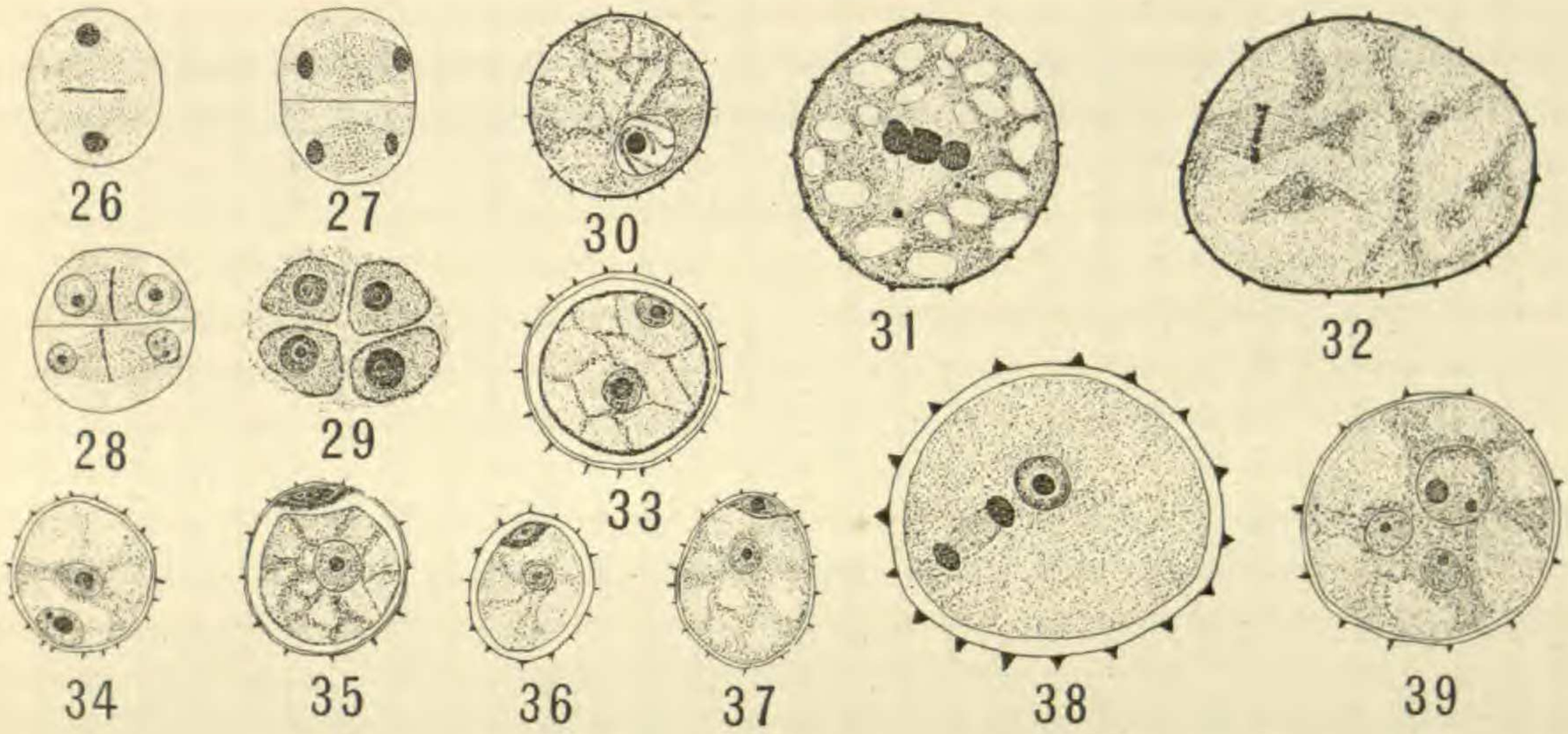
FIGS. 24, 25. Some of the mother cells broken down and lying more or less attached to the tapetum. × 1375.

GERMINATION OF THE MICROSPORE.

The walls of the spore thicken, and, after a short period of rest, the nucleus shows signs of the approaching germination (*fig. 30*). In *fig. 31* is shown a case of division of the microspore nucleus. At the ends of the spindle are distinct granules of unequal size, while near the spindle in the cytoplasm are other granules, apparently of the same nature, so far as appearance and staining reaction can testify. The spores at this time are usually well filled with refractive food masses.

Usually, though not always, the first division results in the placing of one daughter nucleus near the wall of the microspore (*fig. 32*). The nucleus so placed is the nucleus of the generative cell. This cell is small, slightly lenticular in outline, and stains a little more deeply than the large cells of the germinating microspore (*figs. 33–39*). It remains more or less closely applied to the wall of the spore for a short time, then after moving a little distance from the wall divides (*fig. 30*). The spindle of this division is smaller than that of the preceding one,

the resulting nuclei being also smaller (*figs. 38, 39*). By the time these divisions have been completed the outer spore wall is quite heavy, and is covered irregularly with spiny outgrowths.



FIGS. 26–29. Formation of tetrads. × 1375.

FIG. 30. Microspore in which the nucleus indicates approaching germination × 1375.

FIG. 31. First division of the nucleus of the microspore; granules at ends of spindle and in adjacent cytoplasm. × 2400.

FIG. 32. Same as *fig. 31*. Position of spindle which places one daughter nucleus near wall of spore. × 2400.

FIGS. 33–37. Microspores after first division of nucleus; one cell placed near the wall. × 1375.

FIG. 38. Microspore with generative cell dividing. × 2400.

FIG. 39. Same as *fig. 38*, showing male cells. × 2400.

No indications of thinner regions of the spore wall, especially developed to facilitate the escape of the pollen tube, could be found.

Before this time the tissues separating the two loculi on each side have broken down, forming the two pollen sacs. These break open and the spores float out upon the water. The microspores of the two anthers are not in the same stage of development at the same time. When the mother cells of the older anther are beginning to divide, the corresponding cells of the younger anther are just becoming free in the mucilaginous material of the broken down cell walls and tapetum. Conse-

quently the microspores are not discharged from the two anthers of a flower at the same time.

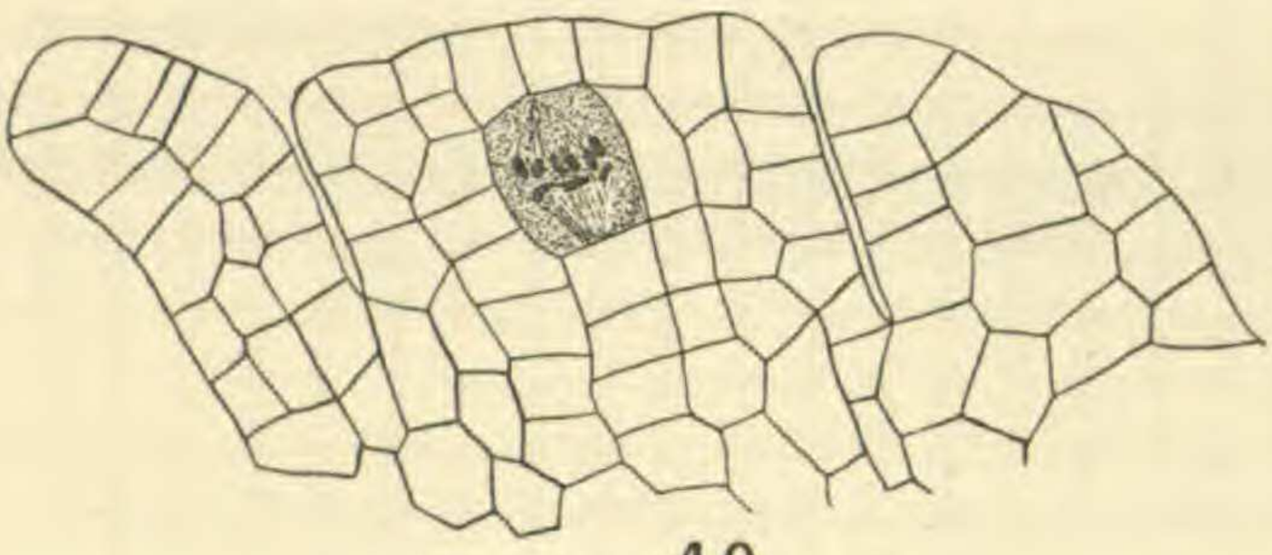
CARPELS.

When the stamens are in the stages of development shown in *figs. 14* and *15*, the beginning of the ovule usually may be distinguished. In *fig. 8* the nucellus appears as the elevation in the center, while the outgrowth shown in section on either side is the beginning of the carpel. The walls of the carpel grow very rapidly, soon extending beyond the tip of the nucellus, at which point they approach each other. Instead of the carpel walls coming together immediately above the nucellus to form a solid style, there is a canal which in the adult carpel extends from one-fourth to one-half the length of the style. The outer end of the carpel is also deeply funnel-shaped, thereby greatly reducing the amount of tissue through which the pollen tube must pass. As the young carpel develops, it presses against the upper wall of the flower pouch. This causes it gradually to change its direction until its longitudinal axis lies almost in the plane of the axis of the stamens (*fig. 9*). When the tip of the carpel escapes from the spathe the funnel-shaped stigma turns upward (*figs. 1, 2*). Whether this is merely an attempt to come to the surface of the water to catch pollen grains, or to extend above the surface to protect the mucilaginous secretion, I could not determine. In either case it seems clear that the deep funnel-shaped tip is well constructed to hold pollen grains which have once become lodged therein.

OVULES.

The axial ovule begins to grow with its apex pointed almost directly toward the surface of the plant, its longitudinal axis being at an angle of about 60° from that of the stamens, which it will be remembered lie in a plane almost parallel to the surface of the frond. The nucellus appears first, as a few-celled papilla, which very early has at its sides the projections from which the integuments develop (*figs. 40, 41*). On one side the

integuments grow much more rapidly than on the other, so that when the ovule is fully formed the embryo sac lies perpendicular to the stalk of the ovule (*figs. 9, 10, 42*), and in a



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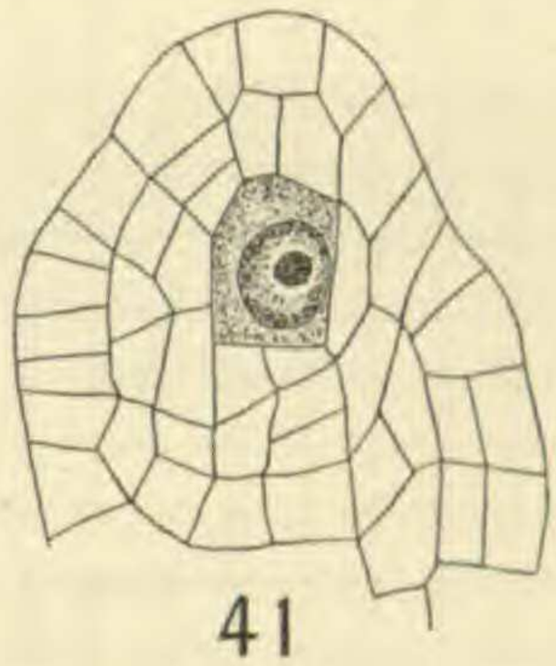

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FIG. 40. Nucellus with dividing archesporial cell; at each side the beginning of the carpel; at rim of nucellus the beginning of first integument. × 1375.

FIG. 41. Nucellus, with megaspore, tapetal cells, and beginning of integuments. × 1375.

plane parallel to the surface of the frond, with its micropylar end toward the base of the frond. It must be borne in mind that while the ovule when young was orthotropous, and gradually became half anatropous, the carpel has also changed from an upright position to one which is prostrate. Thus the position of the ovule has constantly changed in two directions during growth, making it extremely difficult to obtain sections showing the successive stages in the growth of the carpel, ovule, and embryo sac. This difficulty will be more fully appreciated when it is remembered that the flowers are so small that it is impossible to orient them, or to determine their respective ages except under high magnification. As a result of these conditions most of the hundreds of series of sections made were of no great value.

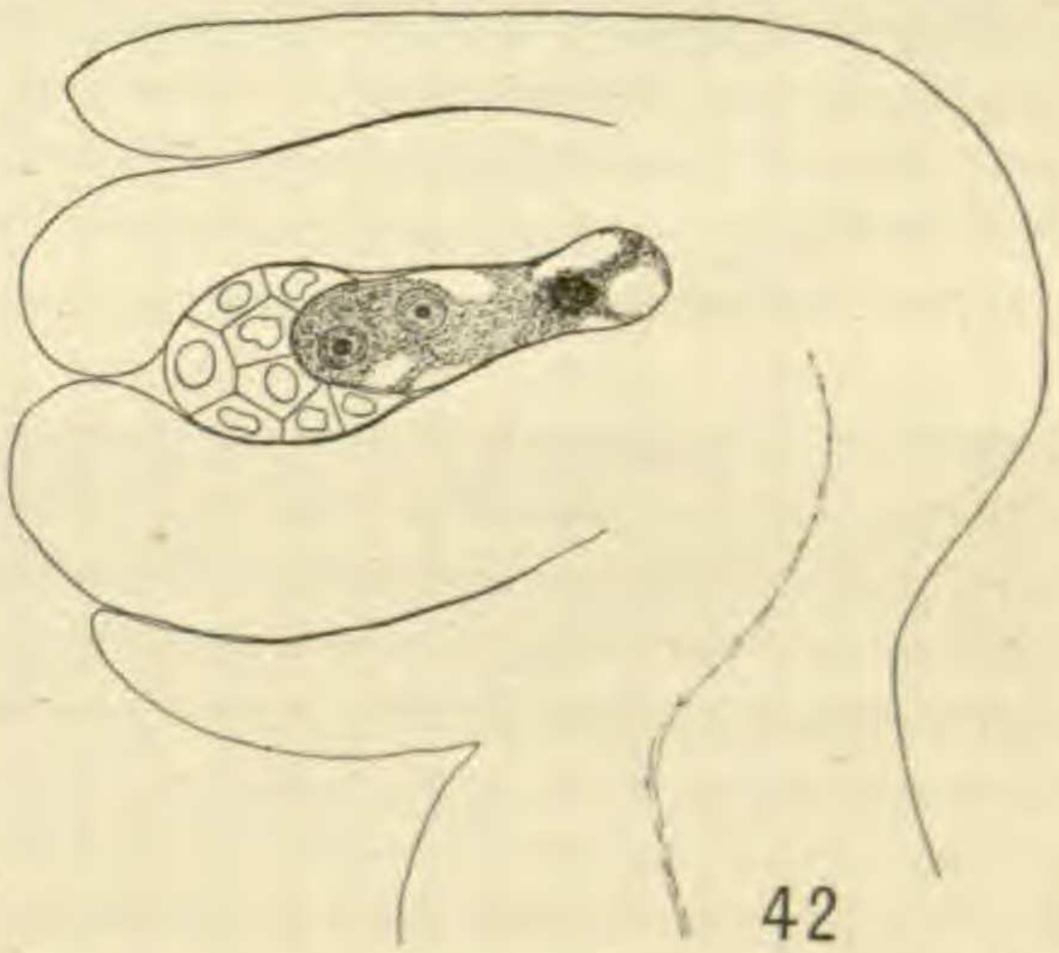

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FIG. 42. Ovule with embryo sac in which antipodal nucleus is disintegrating; the nucellus caps the embryo sac. × 890.

FORMATION OF THE MEGASPORE.

Quite early in the development of the ovule an archesporial cell (*fig. 43*) may be distinguished by its greater size and greater avidity for stains. Its cytoplasm stains much more

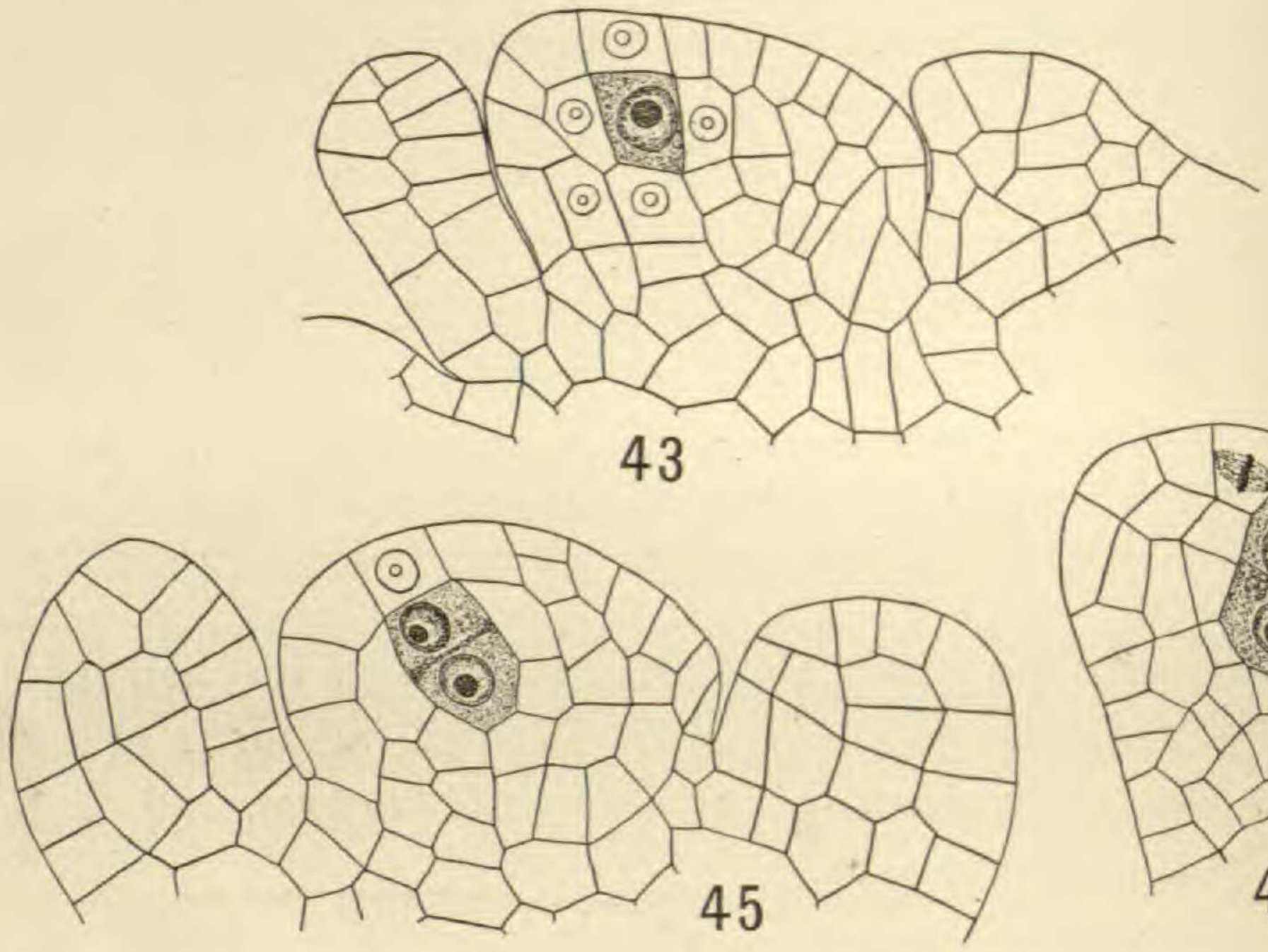


FIG. 43. Nucellus with archesporial cell. × 1375.

FIGS. 44, 45. Same as *fig. 43*, but archesporial cell has divided, forming primary tapetal and primary sporogenous cells. × 1375.

darkly than that of adjacent cells, while its nucleus takes a less intense stain. This cell enlarges rapidly and soon divides (*fig. 40*), giving rise to the primary tapetal cell and the primary sporogenous cell (*figs. 44, 45*), this division usually occurring about when the carpel begins its rapid growth around the ovule (*fig. 45*). The primary tapetal cell may divide by a wall perpendicular to the one which separated it from the primary sporogenous cell (*fig. 41*). I found no cases indicating that it divides by periclinal walls to form a tapetal row. Later stages do not show more than two layers of cells above the tip of the embryo sac, and since nothing was found which could be interpreted as remains of tapetal cells the conclusion that no later development occurs seems justified. The loss of differentiation in reaction to

stains soon renders the tapetal cell indistinguishable from the nucellar cells about it.

The primary sporogenous cell seems to develop directly into the megaspore, and as such undergoes a long period of rest. Meanwhile the integuments push beyond and enclose the nucellus. After the megaspore is formed the cells of the unusually small nucellus undergo no further divisions.

GERMINATION OF THE MEGASPORE.

The enlarging megaspore encroaches upon the nucellus, which, as will be seen from the figures, presently consists of a

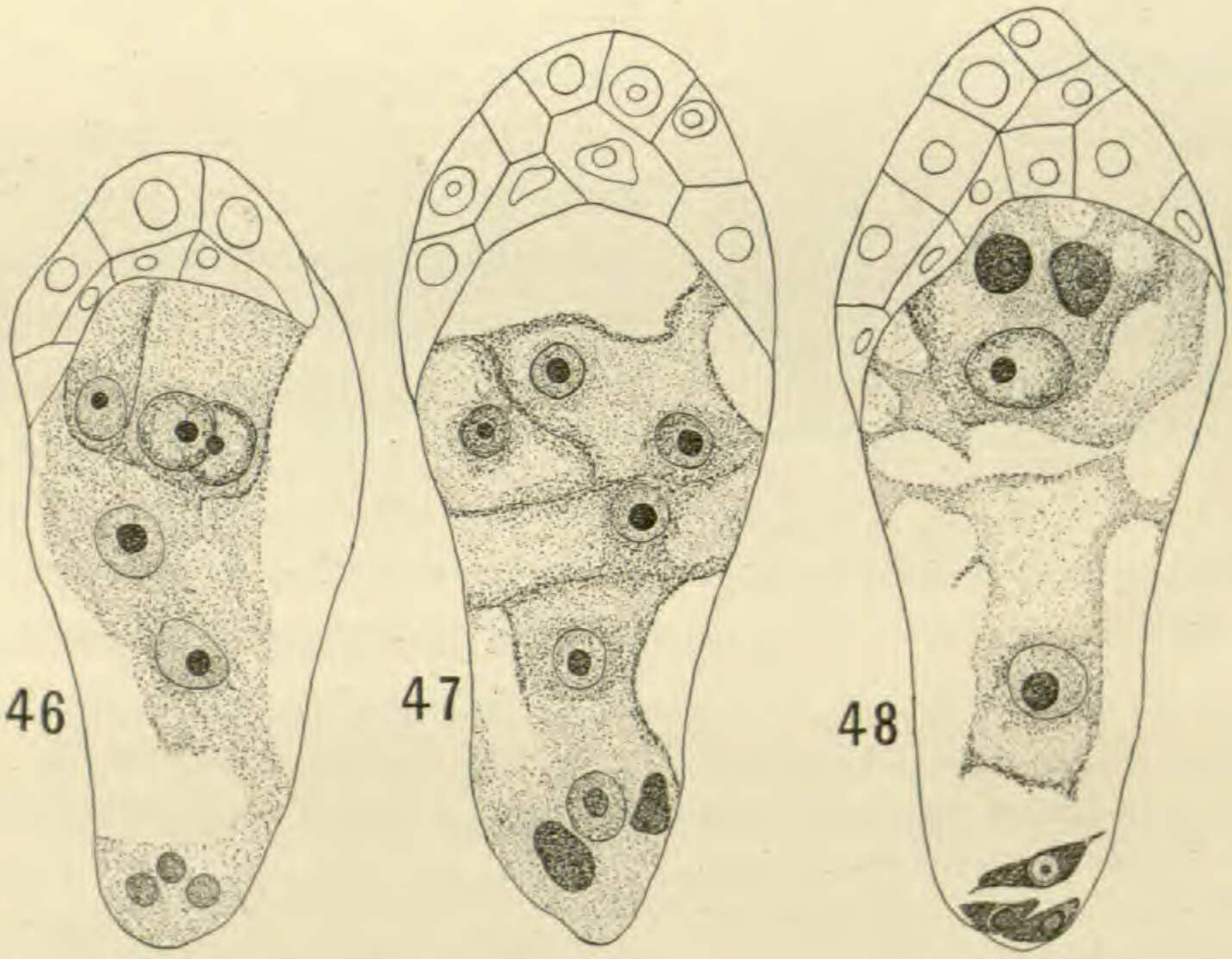


FIG. 46. Eight-celled embryo-sac with nucellus capping micropylar end. × 1375.
FIG. 47. Probably an eight-celled sac. × 1375.
FIG. 48. Sac with oospore, synergids, primary endosperm nucleus, and antipodals. × 1375.

few cells which are so arranged as to form a cap to the growing megaspore. The sides of the megaspore are bounded by the integuments, and its lower end by the chalazal region of the ovule. The nucellus is crowded into the micropyle, and frequently has some of its cells absorbed by the megaspore, although

its outline usually remains rather definite until late stages (*figs. 46, 47, 48, 49, 50*). This same condition was found in *Pistia Stratiotes;* while in *Allium Canadense* (19) the nucellus is said to disappear quite early.

Up to this point the sequence is quite regular, but later there appear many irregularities of such a nature as to indicate general unfavorable conditions for normal development. Although after prolonged search I succeeded in finding preparations showing the usual behavior of the angiosperm embryo sac, these preparations constituted a very small part of those which should be considered in studying the real conditions of the embryo sac of *L. minor.* It is only exceptional cases in which sacs beyond the one-celled stage do not give some evidence of disorganization. The sections selected for drawing do not fully represent these conditions, since they were selected to illustrate normal as well as abnormal occurrences.

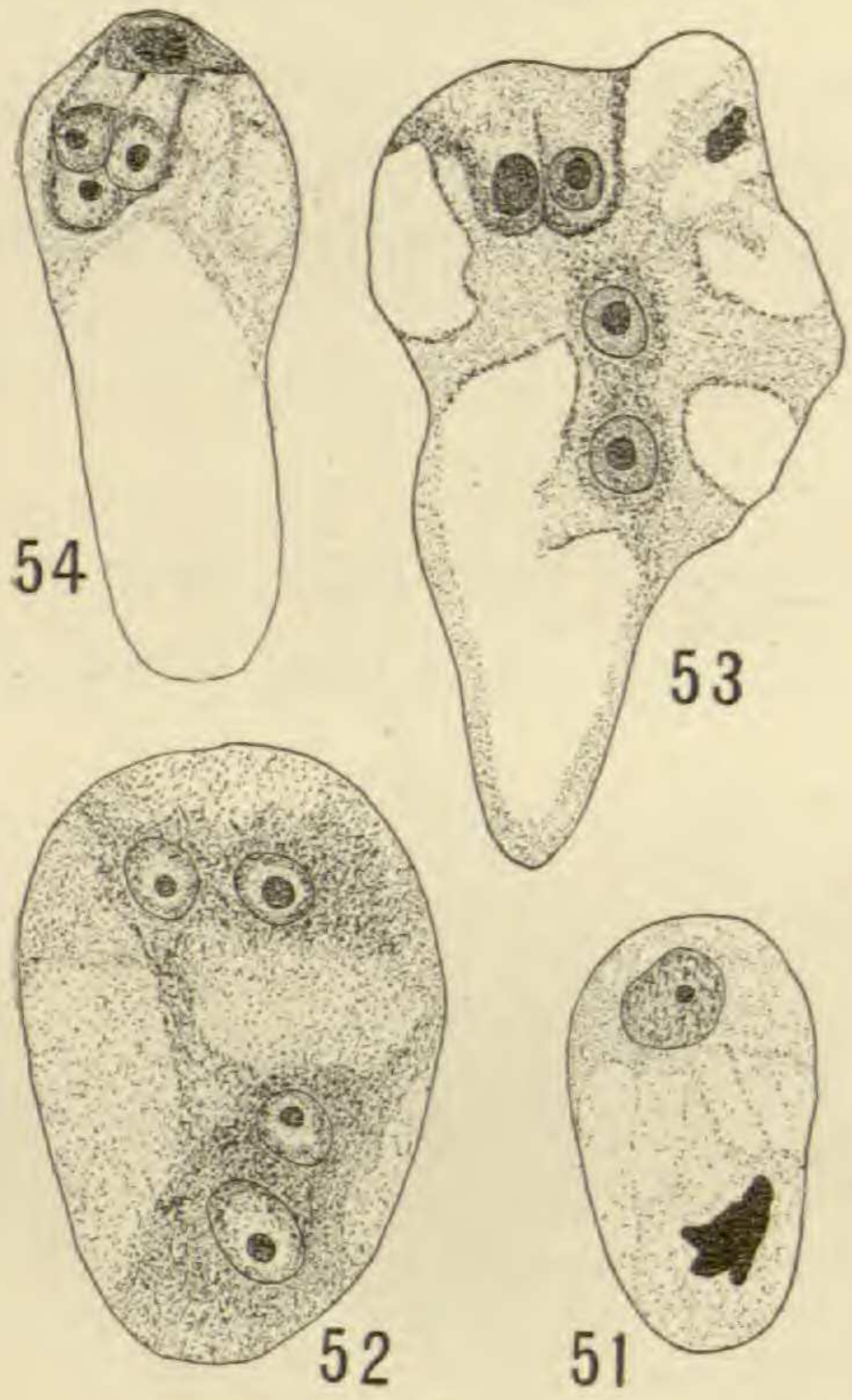


FIG. 51. Two-celled sac in which antipodal nucleus is disintegrating. × 1375.

FIG. 52. Four-celled sac. × 1375.

FIG. 53. Sac in which egg apparatus is formed; probably polar nuclei below egg apparatus. × 1375.

FIG. 54. Sac with egg apparatus and pollen tube. × 1375.

In *fig. 51* is shown a sac in the two-celled stage, in which the micropylar nucleus is normal, while the antipodal nucleus is disintegrating. Both nuclei may again divide, forming the four-celled sac (*fig. 52*), and each of these may divide in the normal manner. It is quite common to have the micropylar nuclei develop normally, while the antipodal nuclei disintegrate (*figs. 42, 46, 51, 53, 54*).

The polar nuclei may fuse to form the primary endosperm

nucleus (*figs. 46, 48, 50*); or they may fail to meet (*fig. 55*), in which case the upper polar nucleus seems to have been able to form endosperm cells without the assistance of the lower one. I was not able to determine whether the two cells below the

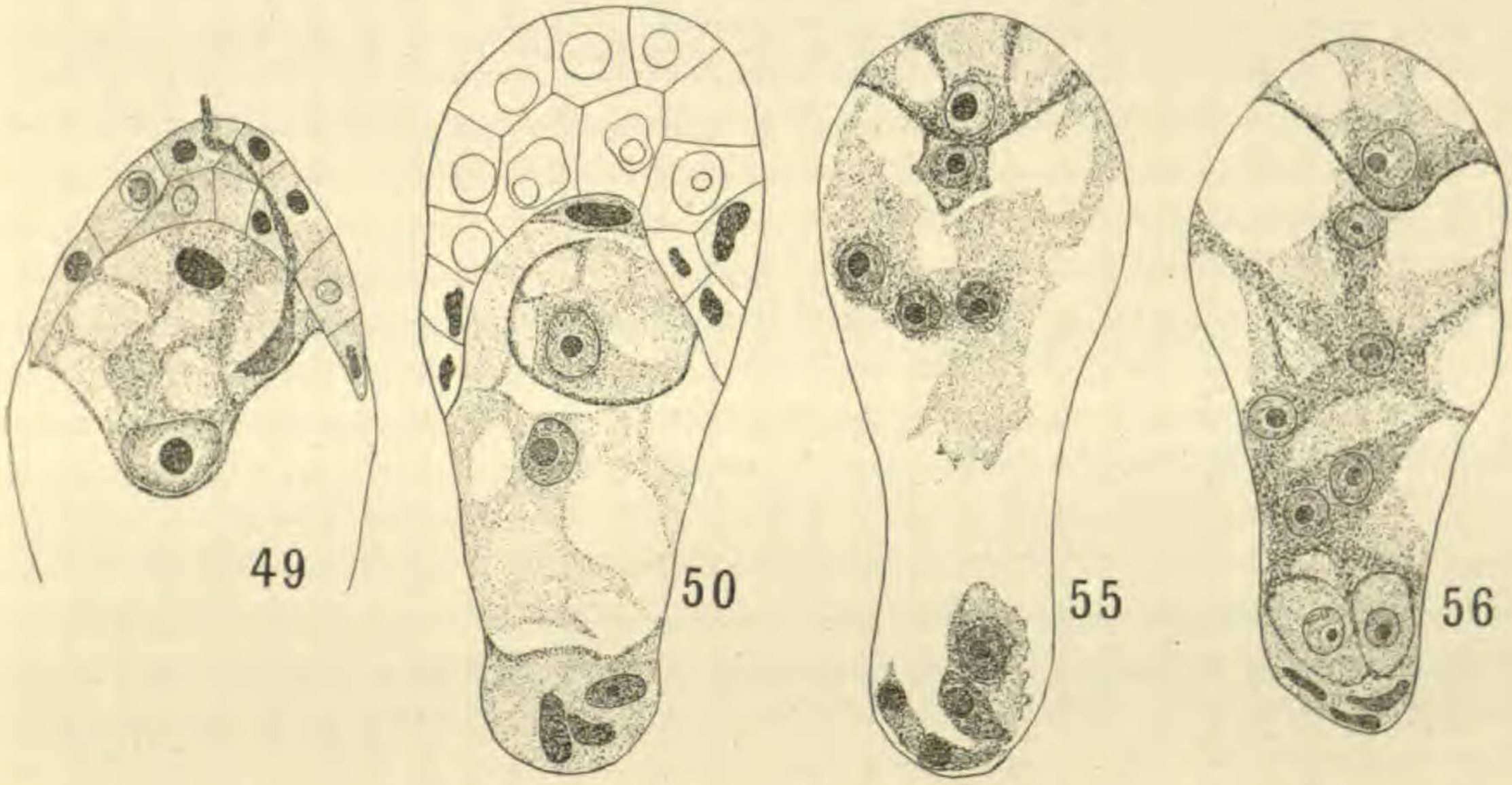


FIG. 49. Micropylar end of sac; recently formed oospore; part of pollen tube in sac and nucellus. × 1375.

FIG. 50. Sac with unusually large oospore. × 1375.

FIG. 55. Sac with oospore, endosperm, and antipodals; lower polar nucleus with antipodals. × 1375.

FIG. 56. Same as *fig. 55*, except the peculiar cells with the antipodals which may have come from division of lower polar nucleus. × 1375.

egg apparatus in *fig. 53* are endosperm cells or polar nuclei. An interesting case is shown in *fig. 56*, in which the upper cell is probably an oospore, while below it are five cells, evidently endosperm cells. In the antipodal end of the sac are the remains of the antipodals, and immediately above them two very large cells which have the cytoplasm somewhat definitely organized. They do not stain as disintegrating cells, but react in a manner quite different from the five cells above them. I am not able to speak confidently in reference to these cells, but it is quite possible that they may have resulted from the division of the lower polar nucleus, while the five cells above may be the progeny of the upper polar nucleus.

Similar behavior is reported for *Allium tricoccum* (19) and *A. Canadense* (19), in which of 170 embryo sacs examined at stages which should show antipodals they were found in but forty-five, there being in some of these but one or two of the cells present, and these very small and irregularly crowded together. Of these 170 sacs the egg apparatus was found in 165, and later stages examined showed normal embryos. A very large number of older ovules of *A. cernuum* was examined, there being but six embryos found.

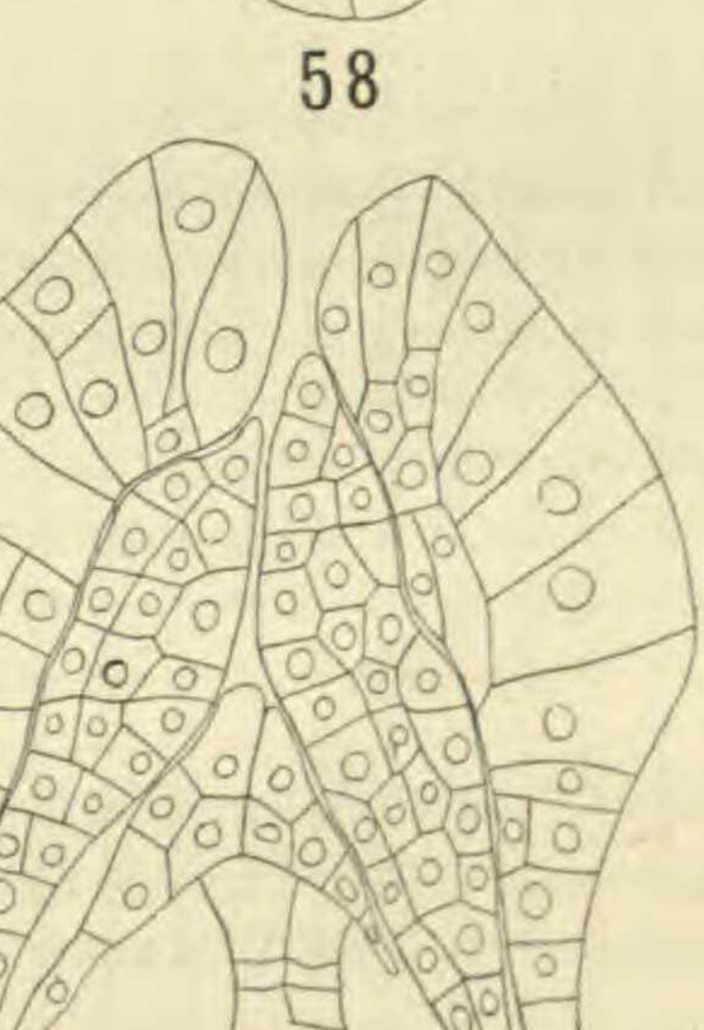
FERTILIZATION AND FORMATION OF EMBRYO.

A very small per cent. of the female gametophytes succeed in developing oospheres and persisting until fertilization occurs, for, as has been stated, disorganization most often occurs before the egg apparatus is complete. The succession of regions as disorganization proceeds is noteworthy. Disorganization first affects the antipodals and may proceed no further, a condition of things very common among angiosperms; it may advance to the polar nuclei or endosperm and stop there; or it may involve the egg apparatus, which is the last to succumb. This process often involves the entire ovule to such an extent that it has almost disappeared when under ordinary conditions oospores would be found.

A sufficient number of cases were found, however, to show that fertilization and embryos may occur. In one case the pollen tube was seen to have penetrated the sac, and extended almost to the oosphere (*fig. 54*), and in *fig. 49* the remnants of the tube appear while the sex cells have just fused. An abnormally large oospore, which almost fills the micropylar end of the sac, is shown in *fig. 50*. Other oospores are shown in *figs. 48, 55, 56*. It is quite evident that many more oospheres are formed than are fertilized.

I was unable to make a sufficiently full investigation of the development of the embryo to justify any definite conclusions. The embryos shown in *figs. 57* and *58* do not show the form supposed to be typical for monocotyledons, as given in Alisma (5 and 13),

and Lilæa (18). But, as in *Lilium Philadelphicum* (15), the suspensor cells have divided by longitudinal walls and no definite embryo cell or cells can be distinguished. It is quite evident that there is more of a suspensor than is shown for this species in Hegelmaier's *Monograph* in his *figs. 3, 4, 5*. Other figures by Hegelmaier represent young embryos of this species as each having a very large terminal cotyledon, with the small plumule arising laterally near the suspensor region.



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After fertilization the cells at the tips of the integuments enlarge and divide (*fig. 59*), crowding close

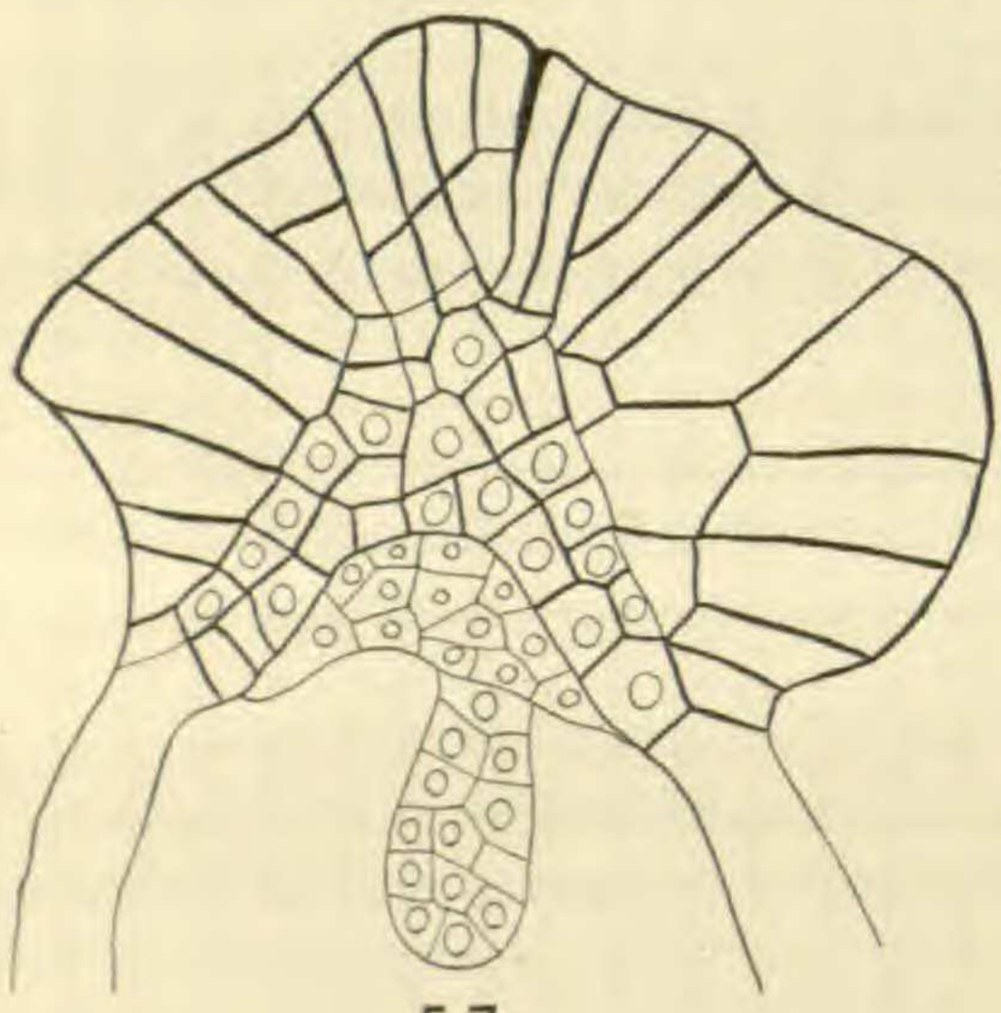

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FIG. 57. Embryo, nucellus, and the peculiarly thickened tips of the integuments. × 760.

FIG. 58. Young embryo attached to the nucellus; the base of the same embryo is shown in *fig. 59*. × 1375.

FIG. 59. Tips of integuments, the cells of which are enlarged; the nucellus and lower end of the embryo. × 760.

together until there is a compact tissue of heavy-walled cells completely closing the micropyle (*fig. 57*), forming the so-called beak or operculum of germinating seeds. In no case where fertilization was not accomplished was such a structure seen.

In undertaking this investigation I had hoped to find abundant embryos and germinating seeds, in order to determine the comparative morphology of the parts of the plant. But as few were found I can make little addition to the statements of other writers. When the seed coats burst the cotyledon appears first, and is so folded that it encloses the young stem. An absorbing organ remains in contact with the food material of the seed. The basal and nodal regions of the shoot soon become differentiated, and quite early there are developed the pouches in which are the new vegetative fronds. The energy of the plant now seems to be directed toward this new frond rather than to the embryonic organs, since all of the latter very soon disappear.

ECOLOGICAL NOTES.

In this connection I need but to mention the numerous large air spaces for aeration and floating, and the chloroplastids which have such great power of adjusting themselves to the light, to recall two prominent ecological adaptations of this plant.

Water and pollination.— It will be remembered that most water plants, *e. g.*, Naias, Vallisneria, and Elodea, have adaptations for securing pollination which are especially suited to their water environment, while the structures of the ovule, so far as reported, are rather normal. In Lemna we seem to have a plant which has exchanged a land for a water habitat, and in so doing has not succeeded in working out such effective devices for pollination as have the real water plants. This might add additional testimony in favor of the view that lemnas are derived from terrestrial forms. The terrestrial method of pollination seems to have proved almost a failure in the new conditions, and this may be the ultimate cause for the more or less complete disorganization of the structures of the embryo sac.

Winter buds.—There has been much discussion with reference to the winter buds, the usual idea being that they are morphologically different from the summer buds, but I have not been able to detect any striking differences. In the winter buds the air spaces

are not abundant, and the bud is more nearly spherical. It must be borne in mind, however, that winter buds are formed only when the environment is becoming unfavorable. For this reason fewer air spaces are developed, and the cells do not divide so as to increase the bud in length. The greater rotundity of the winter bud, therefore, is not due to increase in thickness, but rather to decreased length, as compared with the ordinary summer buds. Since few air spaces are developed and gases are no longer actively produced, when the bud becomes free it sinks to the bottom of the pond or stream, or remains suspended in the water a little above the bottom. When the conditions again become favorable the bud begins to grow while in the winter position, and soon produces sufficient air spaces and gases to cause it to come to the surface of the water. By this time it has usually begun one or two new vegetative fronds.

My observations do not indicate that winter buds necessarily follow the production of flowers as stated by Guppy (10). A large number of cases were observed in which the usual summer frond develops at the same time that the flower is formed, or immediately afterwards. Hegelmaier doubts whether winter buds are ever formed in the tropics.

Flowers.—The ecological significance of the conditions of the flower of *Lemna minor* is very suggestive. It is known that flowers are developed very infrequently, and when one considers that in most of those formed the embryo sac structures and ovules break down at various stages in their development, resulting in great paucity of seeds, it becomes evident that the conditions which favor vegetative multiplication have led to great reduction of ordinary seed formation. The device of winter buds also greatly assists the plant in discontinuing the seed habit.

SUMMARY.

1. The sporophyte of *Lemna minor* cannot be definitely homologized with either a stem or a leaf, but is a shoot undifferentiated except at the basal and nodal regions.

2. The secondary root is formed from a group of hypodermal

cells at the node. The epidermis, which is pushed out, persists for a considerable time as the temporary root-sheath. The root-cap while young adheres to the main body of the root, but later becomes entirely free except at the growing point. The number of cells constituting the meristem region is unusually small, sometimes being reduced to one or two. An axial strand of undifferentiated cells is the representative of the conducting system of the root.

3. Flowers are rarely formed, and when present part or all of their organs may disorganize at any stage in their development.

4. In young flowers the nucellus and stamen papilla first appear, and about the nucellus is the beginning of the carpel. The spathe appears outside of the carpel and stamen papilla. The two stamens arise from the branching of the papilla.

5. A single archesporial mass appears in each stamen. This is later divided into two, then into four masses, constituting the archesporial masses of the four loculi of the anther, which four loculi constitute one and not four sporangia.

6. The primary tapetal layer is not cut off at the first division of the archesporial cells, but after these have become separated into four regions.

7. It seems clear that the tapetum is not a morphological but a physiological layer.

8. After the microspore mother cells have become free the cells of the tapetum frequently divide, pushing into the cavity of the loculus.

9. Many microspore mother cells disintegrate and function as tapetal cells.

10. The microspores germinate within the sporangium. The generative cell remains closely applied to the wall of the spore for a time before dividing.

11. In the megasporangium the primary tapetal cell usually undergoes no further division, while the primary sporogenous cell passes directly over into the megaspore.

12. Normal embryo sac structures are developed in com-

paratively few cases, the rule being that disorganization stops the process at some stage. This disorganization first affects the antipodal end of the sac. In sacs which have succeeded in developing endosperm we find it next attacked; and last to succumb to unfavorable conditions is the egg apparatus.

13. In case the polar nuclei fail to fuse one or both of them may develop endosperm without fusion.

14. Owing to the above conditions there is great paucity of seeds, not enough having been found to determine the homologies of the sporophyte.

15. The winter buds are summer buds which have failed to develop sufficient air spaces and gases to float them, and hence sink. When conditions become favorable again such are developed and they again come to the surface.

16. *L. minor* seems to have descended from terrestrial forms, and has not succeeded in adjusting to a water environment the processes involved in developing seeds.

In closing I wish to express my thanks to Dr. John M. Coulter and Dr. Charles J. Chamberlain for many valuable suggestions and criticisms given during the progress of this work.

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